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Comparative Evaluation of The Cytotoxicity Effect of Orthodontic Miniscrew Implants of Titanium Alloy and Stainless Steel in Different Mouthwashes - An in Vitro Study

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Abstract

Background & Objectives This study aims to evaluate the cytotoxicity effect of orthodontic miniscrerw implants of Titanium alloy and Stainless steel alloy in different types. To compare & evaluate the cytotoxicity of Titanium based miniscrew implants in 0.2% chlorhexidine mouth wash, 0.01% fluoride mouth wash, 2% povidone iodine mouthwashes and distilled water. To compare & evaluate the cytotoxicity of stainless steel based miniscrew implants in 0.2% chlorhexidine mouth wash, 0.01% fluoride mouth wash, 2% povidone iodine mouthwashes and distilled water. To compare & evaluate the cytotoxicity of Titanium based miniscrew implants and stainless steel in 0.2% chlorhexidine mouth wash 0.01% fluoride mouth wash, 2% povidoneiodine mouthwashes and distilled water

Methods: In this invitro study, 84 samples of 4 different mouthwashes were divided into 4 groups which is further divided into 3 subgroups: Two experimental group and one control group(without TAD)The eluates were extracted from mini implants and cultured with human gingival fibroblasts for cytotoxicity assessment using the MTT assay. Cell viability percentages were calculated as (Treated cells/Negative control cells) * 100.

Results: The Titanium alloy & Stainless steel implants exhibited favorable biocompatibility with minimal cytotoxicity with chlorhexidine mouthwash solution tested. In contrast, varying degrees of cytotoxic responses, particularly under povidine iodine and fluoride exposure, attributed to the release of nickel and chromium ions by SS. Statistical analysis revealed significant differences in cytotoxicity profiles between titanium and stainless steel implants, emphasizing the influence of material composition on implant biocompatibility.

Interpretation & Conclusion In this study, CHX proved to be minimal cytotoxicity to fluoride & povidine iodine mouthwashes. So CHX mouth wash is effective in reducing cytotoxicity caused by release of metal ion by OMI.

Keywords: Titanium, stainless steel, Povidone iodine, Chlorhexidine, Cytotoxicity

Introduction

Orthodontic miniscrew implants, well-known as temporary anchorage devices, have changed orthodontic treatment by providing a constant anchorage for tooth movement. These implants are proposed to be temporarily embedded into the bone to serve as stable anchorage, allowing more precise and controlled orthodontic force application. [1] Miniscrew implants, unlike traditional anchorage procedures like headgear or intraoral appliances, provide adaptability and steadiness without the need for patient compliance or cooperation .Therefore, in the last few years, mini implants have been extensively used for anchorage, thus simplifying orthodontic mechanics and minimizing side effects during orthodontic treatment [2].

Recent advances in implant material, design, and placement techniques have led to a substantial use of orthodontic miniscrew implants.[3] Miniscrew implants, which provide predictable and efficient anchorage, have expanded the scope of orthodontic treatment, permitting clinicians to accomplish optimal outcomes in challenging cases [4].

Titanium alloy, which is mainly composed of titanium and also trace amounts of aluminium and vanadium, has excellent biocompatibility, corrosion resistance, and mechanical strength [5]. These properties mark titanium alloy an excellent choice for orthodontic miniscrew implants, as it eases the risk of allergic reactions and adverse tissue responses though maintaining long-term stability and functionality [6].

Stainless steel, known for its exceptional mechanical properties, such as high tensile strength, hardness, and durability, does not have the same level of biocompatibility as titanium alloy, its durability and cost-effectiveness make it a feasible choice for orthodontic miniscrew implants, particularly when high mechanical strength is required [7].

Chlorhexidine, fluoride and povidine iodine mouthwashes are common choices of mouthwashes used in oral hygiene maintenance to reduce plaque accumulation, control bacterial growth, and promote gingival health [8]. The chemical composition of mouthwash, contains antimicrobial agents, fluoride, alcohol, and other additives, have potential to interact with orthodontic materials, including miniscrew implants [9]. Such interactions may affect the surface

properties, corrosion resistance, and biocompatibility, of the implant affecting their clinical performance and longevity[10].

Despite their biocompatible, the cytotoxicity profiles of titanium alloy and stainless steel may vary upon different factors, like surface characteristics, chemical composition, and environmental circumstances [11]. Exposure to mouthwashes adds another variable that could influence the cytotoxicity of these implants, as certain ingredients or additives in mouthwashes can worsen or mitigate cellular responses [12]. Null hypothesis states that there is no significant difference in the cytotoxicity effect between orthodontic miniscrew implants made of titanium alloy and those made of stainless steel when exposed to different mouthwashes. Thus the purpose of this study is to compare the cytotoxic effects of titanium alloy and stainless steel orthodontic miniscrew implants in different mouthwash solutions.

Materials & Methods

Materials used

- **Orthodontic material**

Titanium miniscrew implant 1.3×6 mm (SK Surgical Orthodontic mini-pin head Ti implant),Stainless steel miniscrew implant 1.3×6 mm (SK Surgical Orthodontic mini-pin head Stainless steel implant

- **Equipment**

Eppendorf tubes, 96-well flat bottom plates, Inverted light microscope

- **Microbiological laboratory material**

Fluoride mouthwash- Listerine(Johnson & Johnson, India)Sodium fluoride 0.02%, 0.01% w/v fluoride ion

-Chlorhexidine mouthwash- Hexidine(ICPA, INDIA) Chlorhexidine gluconate 0.2%

-Povidine Iodine mouthwash- Betadine (Win-Medicare, INDIA) Povidine Iodine 2% mint flavour)

-Distilled water (VITSZEE)

-Human primary gingival fibroblast

-Alpha modified eagle medium

-10% of foetal bovine serum

-100 U/ml penicillin

-100 µg/ml streptomycin

-1% Amphotericin B

-100 µl of acidified isopropanol

-ELISA plate reader

-MTT

Sample size calculations

Sample size estimation was done by using g-power software (version 3.0). Sample size was estimated for exact test. A minimum total sample size of 84 was found to be sufficient for an alpha of 0.05, power of 95 %, 0.5 as medium effect size (assessed from a similar study). Sample size was further rounded off to 80 i.e 20 in each group. This invitro study, had 84 samples of 4 different mouthwashes, divided into 4 groups which were further divided into 3 subgroups i.e., two experimental group and one control group (without TAD)

Groupings

In Group 1, Subgroup a: 7 Titanium Mini screw implants in chlorhexidine mouthwash, Subgroup b: 7 Stainless steel Mini screw implant in chlorhexidine mouthwash, Subgroup c: 7 Chlorhexidine mouthwash without TAD- Control group. In Group 2, Subgroup a: 7 Titanium Mini screw implants in Fluoride mouthwash, Subgroup b: 7 Stainless steel Mini screw implant in Fluoride mouthwash, Subgroup c: 7 Fluoride mouthwash without TAD- Control group. Group 3, Subgroup a: 7 Titanium Mini screw implant in Povidine Iodine mouthwash, Subgroup b: 7 Stainless steel Mini screw implant in Povidine Iodine mouthwash, Subgroup c: 7 Povidine Iodine mouthwash without TAD- Control group. In Group 4, Subgroup a: 7 Titanium Mini screw implant in

Distilled water, Subgroup b: 7 Stainless steel Mini screw implant in Distilled water., Subgroup c: 7 Distilled water without TAD- Control group.

Preparation of eluates from Mini screws

Each type of treatment solution (n = 7) was filled in Eppendorf tubes and labelled based on the groupings. The amount of solution was calculated by the ratio of 1 ml for 0.2 g of Mini screw weight according to DIN ED ISO 10271. Fifty-six specimens of eluates were obtained by individually immersing Mini screws in 385 µl of each type of treatment solution and incubating for 28 days at 37ºc. Each type of mouthwash without Mini screw implants was also be incubated for 28 days as a control group.(Figure 1)

Human primary gingival fibroblast cell culture

Human primary gingival fibroblast (HGF) cells culture in alpha-modified eagle medium (α-MEM) ph 7.2 with 10% of fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, and 1% amphotericin B Cultures were maintained at 37ºc, 5% CO_2, and 95% air until reach 70% - 80% confluence.(Figure 2)

In vitro cytotoxicity by MTT assay

Aliquots of 100 µl of HGF cell suspension (4 × 104 cells/ well) were pipetted into 96-well flat-bottom plates and incubated for 48 h to obtain a cell monolayer. After the monolayer growth it was confirmed by an inverted light microscope, the culture medium was removed and 20 µl of each eluate or 20 µl of each treatment solution with 100 µl of fresh culture medium was added to the correspondent well. Each eluate was tested in quadruple and incubated at 37ºc, 5% CO_2, and 95% air for 24 h. Aliquots of 20 µl 2% chlorhexidine gluconate solution and an additional 20 µl of the medium culture was then added to the positive as well as negative control, respectively. After 24 h, the toxic effect of Mini screws were exposed to the mouthwashes and tested against the mouthwash itself by 3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide (MTT) assay. 10 µl aliquots of MTT solution (5 mg/ml) was added to each well and incubate for another 3 h at 37ºc, 5% CO_2, and 95% air. Then, 100 µl of acidified isopropanol (HCL and isopropanol) was added to dissolve the formazan crystals and the absorbance were read by using an enzyme - linked immunosorbent assay (ELISA) plate reader at 600 nm.(Figure 3)

The percentage of cell viability was calculated as

$$\text{Cell viability (\%)} = \frac{\text{Treated cells}}{\text{Negative control cells}} \times 100$$

Statistical Analysis

Data was analysed using Statistical Package for Social Sciences (SPSS) version 21, IBM Inc. Summarized data was presented using Tables and Graphs. Shapiro-Wilk test was used to check which all variables were following normal distribution .Bivariate analyses was performed using one way ANOVA followed by Bonferroni test for post hoc comparison for continuous variable. Level of statistical significance will be set at p-value less than 0.05.

Result

Table1 shows Overall significant difference in the cell viability when compared among four study groups as $p<0.05$. Table 2: The post hoc analysis reveals significant differences in cell viability between certain study groups. Specifically, Group 1 vs. Group 3 and Group 3 vs. Group 4 show statistically significant differences, indicating that Group 3 has a distinct cell viability profile compared to the other groups. The remaining comparisons do not show significant differences, suggesting similar cell viability levels among those groups.

Table 3 and Figure 5: Overall significant differences were observed in mean cell viability levels among four study groups when Titanium miniscrew implant were immersed in chlorhexidine mouthwash. Table 4: The post hoc analysis for Subgroup A reveals that the only significant difference in mean cell viability is between Group 1 and Group 2, with Group 1 showing higher cell viability. Other comparisons between groups do not show significant differences. This indicates that while Group 1 and Group 2 differ significantly, the other groups have similar levels of cell viability in this study.

Table 5 and Figure 6: Overall significant differences were observed in mean cell viabilitity levels among four study groups when Stainless steel miniscrew implant were immersed in chlorhexidine mouthwash. Table 6: Significant differences were seen in means between certain pairs of groups within subgroup B. Specifically, Group 3 tends to have higher means compared to Group 1 and Group 4, while Group 4 tends to have a lower mean compared to Group 1. Rest all the pairs failed to reach the level of statistical significance.

Table 7 and Figure 7: Overall significant differences were observed in mean cell viability levels among four study groups. Table 8: Significant differences were observed in means between certain pairs of groups within subgroup C. Specifically, Group 3 tends to have higher means compared to the other groups, while Group 4 also shows some significant differences in mean compared to Group 1. Rest all the pairs failed to reach the level of statistical significance.

Discussion

Orthodontic miniscrew implants made of stainless steel and titanium alloy exhibit significant cytotoxicity when exposed to povidone-iodine and fluoride mouthwashes, leading to the rejection of the null hypothesis. This study aims to compare the cytotoxic effects of orthodontic miniscrew implants made of titanium alloy and stainless steel when exposed to different mouthwash solutions.

Research by García-Caballero et al. (2015)[14] has shown that chlorhexidine gluconate can significantly reduce cell viability and induce apoptosis in human gingival fibroblasts. Similarly, Goldschmidt et al. (2004) demonstrated dose-dependent cytotoxic responses in gingival fibroblasts following exposure to chlorhexidine mouthwash, highlighting the need for careful monitoring of its usage to prevent potential tissue damage [15].

Studies have shown that sodium fluoride, commonly found in mouthwashes, can induce DNA damage and apoptosis in human gingival fibroblasts [16]. Kato et al. [17] further demonstrated that exposure to fluoride at concentrations exceeding typical mouthwash formulations can lead to significant cytotoxic effects, including altered cellular morphology and reduced cell proliferation.

Povidone-iodine is another potent antimicrobial agent used in various medical and dental applications. Saber et al. (2020)[18] investigated the cytotoxic effects of povidone-iodine on oral mucosal cells and found that high concentrations of povidone-iodine can induce significant cytotoxicity, including cell death and morphological changes in oral fibroblasts and epithelial cells.

The stability of Ti under corrosion conditions is essentially due to the formation of the stable and tightly adherent thin protective oxide layer on its surface[19]. Ammar et al. (2021) stated that titanium alloys are generally biocompatible and exhibit minimal cytotoxic effects on human gingival fibroblasts (HGF) and other oral cells[20]. This supports the current study's finding of relatively low cytotoxicity for titanium alloys.

Since the Ni atoms are not strongly bonded to form some intermetallic compound, the likelihood of in vivo slow

Ni ion release from the alloy surface is increased, which may have implications for the biocompatibility of these alloys[21,22,23]. Similarly our study findings revealed that stainless steel orthodontic miniscrew implants have shown comparable cytotoxic profiles to titanium alloys.

Overall significant differences were observed in mean cell viability levels among four study groups when Titanium miniscrew implant were immersed in chlorhexidine mouthwash, $p=0.013(p < 0.05)$. The post hoc analysis for Titanium miniscrew implant reveals that the only significant difference in mean cell viability is between Group I and Group II, with Group I showing higher cell viability. Other comparisons between groups do not show significant differences. This indicates that while Group I and Group II differ significantly, the other groups have similar levels of cell viability in this study.

Wulan Utami et al. (2022) study stated that chlorhexidine and fluoride mouthwashes increased cytotoxicity due to corrosion products released from titanium orthodontic miniscrew implants. [24]

Aboodi et al[25], stated that fluoride solution can trigger the corrosion of OMI Ti alloy in the form of sign of corrosion by dots and niches. Previous research by Anwar et al. on the effect of fluoride on the corrosion properties of commercially pure titanium (cp Ti) and titanium alloy dental implants revealed that using fluoride therapy (NaF) above 0.1 M significantly decreases the corrosion resistance of Ti and Ti alloy[26].

Povidone-iodine (PI) affects the corrosion behavior of Ti-1 by increasing cathodic current density and decreasing anodic current density, which shifts the corrosion potential anodically. These findings underscore the importance of concentration control in clinical applications to prevent titanium implant degradation[27].

Our findings, we investigated the cytotoxic effects of 2% chlorhexidine on titanium implants and observed significant toxicity. This contrasts with findings from other studies that typically report lower cytotoxicity for chlorhexidine compared to other antiseptics like hydrogen peroxide and povidone-iodine[28].

There is statistically significant difference in comparison the mean cell viability among the study groups for subgroup B is $p=0.006(p < 0.05)$.Significant differences were seen in means between certain pairs of groups within subgroup B (SS OMI). Specifically, Group III tends to have higher means compared to Group I and Group IV, while Group IV tends to have a lower mean compared to Group I. Rest all the pairs failed to reach the level of statistical significance.

In this study, stainless steel orthodontic miniscrew implants exhibited significant cytotoxicity when exposed to fluoride mouthwashes. This aligns with findings by Espinar et al., who reported that fluoride in mouthwashes can lead to the corrosion of stainless steel orthodontic wires, resulting in the release of nickel and chromium ions, which are cytotoxic.[29]

Povidone-iodine can cause corrosion in stainless steel, although the severity can vary based on factors such as concentration and exposure time. Our study demonstrated significant cytotoxicity of 2% povidone-iodine on human gingival fibroblasts, evidenced by reduced cell viability in MTT assay. Overall significant differences were observed in mean cell viability levels among four study groups, $p=0.003$ Significant differences were observed in means between certain pairs of groups within subgroup C. Specifically, Group III tends to have higher means compared to the other groups, while Group IV also shows some significant differences in mean compared to Group I

The in vitro nature of the study may not fully replicate the dynamic and multifaceted oral environment encountered in vivo, potentially limiting the generalizability of our findings to clinical practice. Identify areas for further investigation, such as exploring underlying mechanisms of cytotoxicity, validating findings in in vivo models, and developing clinical guidelines for mouthwash use with orthodontic implants.

Conclusion

On Cytotoxicity Evaluation of Mouthwash Solutions, Chlorhexidine mouthwash (0.2%) demonstrated minimal cytotoxic effects on cell viability around titanium and stainless steel based miniscrew implants. Fluoride mouthwash (0.01%) showed moderate effects on cell viability, with titanium generally exhibiting higher viability compared to stainless steel. Povidone iodine mouthwash (2%) exhibited significant effects on cell viability, especially around titanium implants, suggesting higher cytotoxicity compared to chlorhexidine.

Comparison of Titanium and Stainless Steel Implants, in chlorhexidine and fluoride mouthwashes, titanium implants generally exhibited higher cell viability compared to stainless steel counterparts, highlighting titanium's biocompatibility advantage.

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Legend Tables, Figures and Graphs

Table 1: Intergroup Comparison of Cell Viability

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group 1	21	95.9195	3.70315	.80809	94.2338	97.6051	89.23	100.00
Group 2	21	86.1785	8.65079	1.88776	82.2407	90.1163	65.14	100.00
Group 3	21	79.0321	11.36771	2.48064	73.8576	84.2067	62.33	100.00
Group 4	21	86.9823	12.36931	2.69920	81.3518	92.6127	61.07	100.00
P Value								0.001*

Table 2: Post Hoc Comparison of Cell Viability of Study Groups

Group	Comparitive Group S	Mean Difference (I-J)	Std. Error	P Value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	2.0	.013190	.020230	.915	-.03989	.06627
	3.0	.061571*	.020230	.016	.00849	.11465
	4.0	-.004429	.020230	.996	-.05751	.04865
2.0	1.0	-.013190	.020230	.915	-.06627	.03989
	3.0	.048381	.020230	.087	-.00470	.10146
	4.0	-.017619	.020230	.820	-.07070	.03546
3.0	1.0	-.061571*	.020230	.016	-.11465	-.00849
	2.0	-.048381	.020230	.087	-.10146	.00470
	4.0	-.066000*	.020230	.009	-.11908	-.01292
4.0	1.0	.004429	.020230	.996	-.04865	.05751
	2.0	.017619	.020230	.820	-.03546	.07070
	3.0	.066000*	.020230	.009	.01292	.11908

*. The mean difference is significant at the 0.05 level.

Table 3: Comparison of Mean Cell Viability among Study Groups for Subgroup A

	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Group 1	95.0563	3.09184	.89254	93.0918	97.0207	89.71	100.00
Group 2	83.2164	12.37622	4.37566	72.8696	93.5632	65.14	100.00
Group 3	84.1457	13.75876	7.94363	49.9671	118.3244	75.33	100.00
Group 4	94.7323	3.67053	1.64151	90.1747	99.2898	90.88	100.00
P Value							0.013*

Table 4: Post Hoc Comparison for Subgroup A

Group	Comparitive Group S	Mean Difference (I-J)	Std. Error	P Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Group 1	Group 2	11.83987*	3.73823	.020	1.5276	22.1522
	Group 3	10.91053	5.28666	.194	-3.6733	25.4944
	Group 4	.32400	4.35949	1.000	-11.7021	12.3501
Group 2	Group 1	-11.83987*	3.73823	.020	-22.1522	-1.5276
	Group 3	-.92935	5.54470	.998	-16.2250	14.3663
	Group 4	-11.51588	4.66905	.091	-24.3960	1.3642
Group 3	Group 1	-10.91053	5.28666	.194	-25.4944	3.6733
	Group 2	.92935	5.54470	.998	-14.3663	16.2250
	Group 4	-10.58653	5.98117	.312	-27.0862	5.9132
Group 4	Group 1	-.32400	4.35949	1.000	-12.3501	11.7021
	Group 2	11.51588	4.66905	.091	-1.3642	24.3960
	Group 3	10.58653	5.98117	.312	-5.9132	27.0862
*. The mean difference is significant at the 0.05 level.						
a. subgroups = subgroup a						

Table 5: Comparison of Mean Cell Viability among Study Groups for Subgroup B

	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Group 1	97.1061	4.41318	1.97363	91.6264	102.5858	89.23	99.36
Group 2	88.3801	5.43500	2.21883	82.6764	94.0838	84.34	98.97
Group 3	81.3516	11.85657	3.42270	73.8182	88.8849	63.52	100.00
Group 4	94.8770	2.16739	.96929	92.1858	97.5682	91.03	96.24
P Value							0.006

Table 6: Post Hoc Comparison for Subgroup B

Group	Comparitive Group S	Mean Difference (I-J)	Std. Error	P Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Group 1	Group 2	8.72600	5.23056	.362	-5.7031	23.1551
	Group 3	15.75456*	4.59792	.011	3.0707	28.4384
	Group 4	2.22912	5.46314	.977	-12.8415	17.2998
Group 2	Group 1	-8.72600	5.23056	.362	-23.1551	5.7031
	Group 3	7.02856	4.31899	.383	-4.8858	18.9430
	Group 4	-6.49688	5.23056	.607	-20.9259	7.9322
Group 3	Group 1	-15.75456*	4.59792	.011	-28.4384	-3.0707
	Group 2	-7.02856	4.31899	.383	-18.9430	4.8858
	Group 4	-13.52544*	4.59792	.034	-26.2093	-.8416
Group 4	Group 1	-2.22912	5.46314	.977	-17.2998	12.8415
	Group 2	6.49688	5.23056	.607	-7.9322	20.9259
	Group 3	13.52544*	4.59792	.034	.8416	26.2093
*. The mean difference is significant at the 0.05 level.						
a. subgroups = subgroup b						

Table 7: Comparison of Mean Cell Viability among Study Groups for Subgroup C

	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Group 1	97.0257	4.84187	2.42094	89.3212	104.7302	89.87	100.00
Group 2	87.6767	5.19363	1.96301	82.8734	92.4800	84.34	98.97
Group 3	71.8365	6.41660	2.61957	65.1027	78.5703	62.33	77.10
Group 4	79.8711	13.48743	4.06661	70.8101	88.9320	61.07	100.00
P Value							0.003*

Table 8: Post Hoc Comparison for Subgroup C

Group	Comparitive Group S	Mean Difference (I-J)	Std. Error	P Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Group 1	Group 2	9.34900	6.07843	.432	-7.4190	26.1170
	Group 3	25.18919*	6.25992	.003	7.9205	42.4578
	Group 4	17.15465*	5.66231	.028	1.5346	32.7747
Group 2	Group 1	-9.34900	6.07843	.432	-26.1170	7.4190
	Group 3	15.84019*	5.39537	.034	.9565	30.7239
	Group 4	7.80566	4.68884	.363	-5.1290	20.7403
Group 3	Group 1	-25.18919*	6.25992	.003	-42.4578	-7.9205
	Group 2	-15.84019*	5.39537	.034	-30.7239	-.9565
	Group 4	-8.03454	4.92183	.380	-21.6119	5.5429

Group 4	Group 1	-17.15465*	5.66231	.028	-32.7747	-1.5346
	Group 2	-7.80566	4.68884	.363	-20.7403	5.1290
	Group 3	8.03454	4.92183	.380	-5.5429	21.6119
*. The mean difference is significant at the 0.05 level.						
a. subgroups = subgroup c						

Figure 1: Preparation of eluates from Mini screws

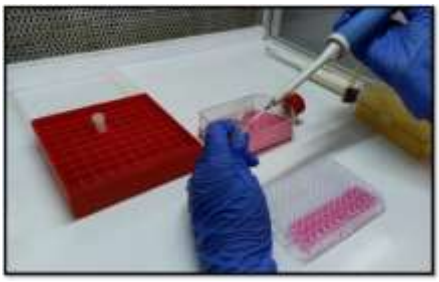
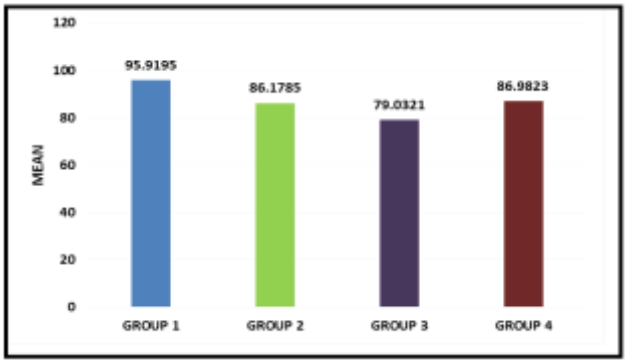
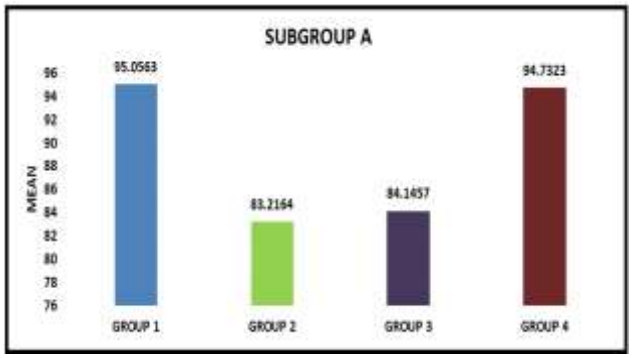


Figure 2: Human primary gingival fibroblast cell culture

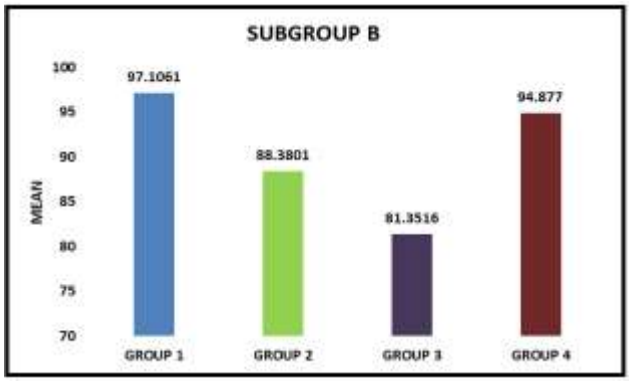
Figure 3: In vitro cytotoxicity by MTT assay



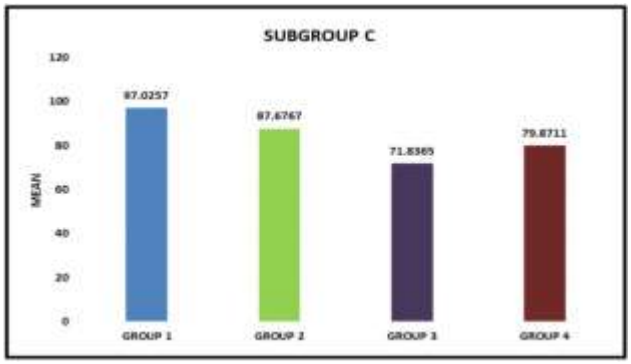
Graph 1: Intergroup comparison of cell viability



Graph 2: Comparison of mean cell viability among study groups for subgroup A



Graph 3: Comparison of mean cell viability among study groups for subgroup B



Graph 4: Comparison of mean cell viability among study groups for subgroup C