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The effects of two forms of commercially available denture adhesives on the growth of Candida albicans in vitro

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ABSTRACT

Introduction

There has been an increased interest in the influence of denture adhesives on Candida albicans growth in denture wearers.

Aims and objectives

To compare the mean colony count and pH of Candida cultures grown in the presence of acrylic resin plates treated with two forms of a denture adhesive.

Design

A quasi-experimental design.

Methods

Two of three groups of 30 acrylic resin specimens were treated separately with two forms of a denture adhesive. The remaining group was left untreated. Ten specimens from each group were incubated for 6 hours, 24 hours and 48 hours following the addition of a diluted strain of *Candida albicans.* The number of colonies and pH of the culture media were recorded over three measurement occasions and analysed.

Results

Plates from the two forms of a denture adhesive had no colonies in the countable range in contrast to plates from the control group. The study found significant mean differences in pH values over three measurement occasions as well as significant group differences in terms of how the means vary over time. However, the results showed a significant time x treatment group interaction.

Conclusions

Both forms of the denture adhesive tested inhibited the growth of candida.

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INTRODUCTION AND BACKGROUND

A large and growing body of literature has investigated the influence of denture adhesives on *Candida albicans (C. albicans)* growth in complete denture wearers.[1-8] A tenuous link between denture adhesive use and denture stomatitis, the leading cause of which is *C. albicans* is suspected. The predisposing factors for denture stomatitis have been widely investigated. These can be categorised into denture wearer's characteristics; behaviour; medical history, and denture-related factors. Predisposing factors such as the denture wearer's age[9], gender[10] and salivary pH[11], behaviours such as high sugar intake[12] and smoking[13] as well as history of systemic diseases such as diabetes mellitus[14], xerostomia[15], hypertension[16], immunosuppressive therapy[17] and extended systemic antibiotic treatment[18] have been researched. The denture-related factors include the propensity of Candida to adhere to the denture base[19], the age of the dentures[20], poor denture hygiene[16,21], and sleeping with dentures.[10,21,22] The prevalence of denture stomatitis has been reported to range between 25% to 40%.[9,10,23,24]

Several studies investigating the in vivo effect of denture adhesives on *C. albicans* growth in denture wearers suffering and those not suffering from denture stomatitis have been carried out.[1,3,6,25-26] These have entailed counting the number of *C. albicans* colonies in saliva and swabs of dentures. Inconsistent results have been reported - whereas Scher et al (1978) reported a reduction in *C. albicans*, Borole et al (2016) found a statistically insignificant increase.[1,25] Kim et al (2003) reported that using a denture adhesive neither increased nor decreased the growth of Candida species.[3]

A great deal of the previous in vitro work in this field has described the adhesion and biofilm formation of *C. albicans* to denture base exposed to denture adhesives as well as measured the mean pH and number of colonies of Candida cultures grown in acrylic resin plates exposed to denture adhesives and incubated for varying periods of time.

Previous studies have reported that denture bases exposed to denture adhesives increased the adhesion and biofilm formation of *C. albicans*.[2,27-28] This increase was however not statistically significant. Darwish et al (2021) demonstrated this increase using denture bases manufactured using UDMA and PMMA resins. The difference in *C. albicans* count between bases manufactured using UDMA and PMMA resins was not statistically significant.[2]

Some studies have found that denture adhesives possess antifungal activity attributed to their effect on the pH of candida culture media.[4,7] Makihira et al (2001) reported that half of the six adhesives tested significantly suppressed the growth of candida species.[4]

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Several studies have reported that denture adhesives lower Candida growth rate in growth curves obtained in the presence of adhesives[8] or reduce the number of Candida colonies in Candida in resin specimen exposed to adhesives.[7,29]

The awareness and use of denture adhesives by denture wearers in South Africa has not previously been described. However, our experience at a tertiary dental teaching hospital in Gauteng indicate that patients use Corega powder or cream. There has been an increasing interest in the influence of Corega on the growth of *C. albicans*. The research to date has tended to focus on the influence of Corega on the adhesion of *C. albicans* to denture bases[2] as well as the growth rate/ number of *C. albicans* colonies in acrylic resin plates exposed to Corega[8] rather than on the influence of Corega on the pH of candida culture media. This is an important issue to research. This study was designed to determine the effect of Corega on the pH of candida culture media.

OBJECTIVES OF THE STUDY

To determine and compare the mean colony count and pH of candida cultures grown in the presence of acrylic resin plates treated with two forms of Corega over three measurement occasions

To determine whether there are significant mean differences in pH values over three measurement occasions
To determine whether there are group differences in terms of how the pH means vary over time.

MATERIALS AND METHODS

Study design

This was a post-test only non-equivalent control group design (a quasi-experimental design).

Study population

The study population consisted of acrylic resin plates

Sample size

The sample size was determined with reference to previous studies.[2,30] Ninety acrylic resin sheets (10 x 10 x 2mm) were prepared according to the manufacturer's instructions and kept in a flask containing normal saline and sterilized in an autoclave at 121^{0}C in line with a method described in a previous study.[31]

Allocation method

A non-random allocation method was implemented i.e., sampling units (sterile acrylic resin plates) were assigned by alternation - one to experimental group A, one to experimental group B, and one to experimental group C.

Interventions

The experiment was conducted according to the procedure used by Rajaram and Manoj (2017), with slight modifications.[7] An inoculum of the cultures of *C. albicans* strain ATCC 90028 was prepared by suspending colonies in 20ml of sterile normal saline and adjusting the turbidity to 0.5 McFarland standard, equivalent to 1.5 × 10^8 forming units. 1:10 serial dilutions of the 0.5 McFarland standard until 10^5 were prepared in sterile saline.

Ninety sterile acrylic resin plates were assigned to three groups of thirty. The sampling groups were labelled as follows: experimental group A, experimental group B, and experimental group C. An amount of 0.011g of Corega cream or powder was lightly applied to each acrylic plate in their respective groups. This amount (0.011g) was decided on with reference to a previous study.[5] No adhesive was applied to acrylic plates in the control group. The acrylic plates in their respective groups were individually placed at the flat bottom of a 25cm^2 tissue culture bottle. Fifty microlitres (0.050ml) of a wide range of dilutions (10^5, 10^3 and 10^2) of the *C. albicans* strain ATCC 90028 was added to each culture bottle and incubated at 37^{0}C for 1 hour to allow C. albicans to seed on the acrylic plates, under aerobic conditions. After the 1-hour incubation period, 2ml of Sabouraud dextrose broth was carefully dispensed into all the culture bottles, and each group was then divided into three equal subgroups i.e., three groups of ten, which were incubated at 37^{0}C for 6 hours, 24 hours and 48 hours respectively.

The pH of the resulting Candida broth cultures was measured at the end of the incubation periods using a calibrated pH meter. Repeated measurements were performed for a random sample of 20% of the cultures.

A calibrated nichrome inoculating loop was used to transfer 0.001ml of the Candida broth cultures (the 10^2 dilution was plated) onto separate Sabouraud agar plates at the end of the incubating periods. Streaks were produced on the agar plates using the loop, after which the plates were incubated at 37^{0}C for 24 hours. The resulting colonies were counted manually as colony forming units (cfu) by the trained principal investigator. The recommendations of Oregon State University were followed: only plates with 25-250 colonies were used; counts above 250 were considered Too Numerous To Count (TNTC) because it was impossible to tell whether colonies were separated, and plates with less than 25 colonies were deemed not to have a statistically significant number of colonies.[31] Repeated measurements were performed for a random sample of 20% of the acrylic plates. The research supervisors denied the principal investigator information that could identify the study groups.

Primary outcomes

The primary outcome measures were the mean colony counts and mean pH values. The effects of interest were the differences in mean colony counts and mean pH values over three measurement occasions between acrylic plates treated with two forms of a commercially available denture adhesive.

Definition of terms

Group A refers to acrylic resin plates which were treated with Corega cream
Group B refers to acrylic resin plates which were treated with Corega powder
Group C is a control group that refers to acrylic resin plates that were not be treated with any adhesive
Sampling unit refers to an individual acrylic plate
Time 1 refers to an incubation period of 6 hours
Time 2 refers to an incubation period of 24 hours
Time 3 refers to an incubation period of 48 hours

Data analysis

Collected data were subjected to univariate and multivariate analysis in Statistical Package for the Social Sciences (SPSS) software version 29. Measures of central tendency and dispersion were calculated.

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A two-way ANOVA (Analysis of Variance) was performed to test whether there are significant mean differences in pH values over three measurement occasions, as well as whether there are group differences in terms of how the means vary over time. The assumptions of two-way ANOVA were checked. These are: (1) inde endence of variables, (2) normal distribution of variables, (3) no outliers and sphericity i.e. constant variance across time points. Bonferroni adjusted pairwise t-tests were performed after significant effects were found. The significance level of the tests was a p-value less than 0.05.

ETHICAL CONSIDERATION

Ethical approval for the study was granted by the Ethics Committee of the institution (SMUREC/D/215/2023:PG). Permission to conduct the study was granted by the Chief Executive Officer (CEO) of the tertiary dental teaching hospital.

RESULTS

Colony counts and pH values recorded from cultures of *C. albicans* incubated at varying periods were analysed. Figure 1 below is a flow diagram of the progress through the phases of the study (that is, enrolment, treatment allocation, follow-up, and data analysis).

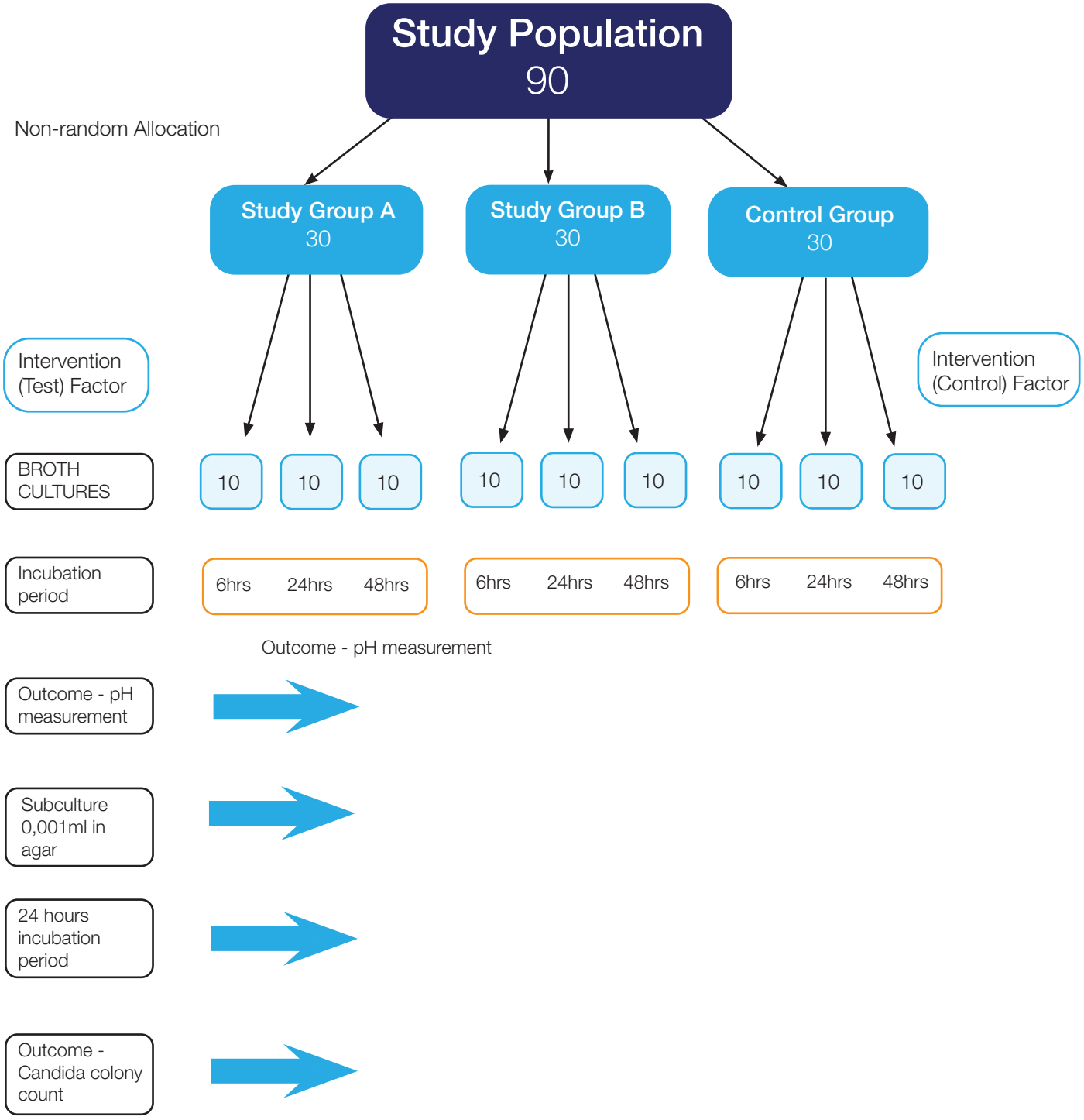

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Table 1: The results of a growth curve of C. albicans grown at 37°C on Sabouraud broth

Time (hours)	Treatment group	Average cfu/ml
6	Corega cream	*
	Corega powder	*
	Control	*
	Total	
24	Corega cream	*
	Corega powder	*
	Control	4,6 x10^7
	Total	
48	Corega cream	**
	Corega powder	**
	Control	**
	Total	

*Statistically insignificant number of colonies.
** Too numerous to count

Not a single plate from any of the treatment groups had colonies in the countable range (25-250) during time 1 (6hours) as well as for the Corega cream and powder groups during time 2 (24 hour) from the 10^2 dilution.

A few number of plates either had a statistically insignificant number of colonies or had colonies that were too numerous to count during time 3 (48 hours). Consequently, average cfu/ml could not be calculated.

Table 2: Descriptive statistics of pH values for each group at each time interval

Time (hours)	Treatment group (tx.group)	Mean pH	Std. dev	Sample size (n)
6	Corega cream	7,0200	,10965	10
	Corega powder	7,1180	,21369	10
	Control	6,9290	,11949	10
	Total	7,0223	,16880	10
24	Corega cream	7,2200	,24490	10
	Corega powder	7,2360	,06150	10
	Control	7,2330	,04620	10
	Total	7,2297	,14318	10
48	Corega cream	,72280	,11612	10
	Corega powder	,72140	,17109	10
	Control	6,9740	,14470	10
	Total	7,1387	,18392	10

The mean pH of Corega powder was the highest during times 1 and 2. The mean pH of Corega cream was the highest during time 3.

Table 3: Output from two-way ANOVA (Mauchly's Test of Sphericity)

Within-Subject Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt
Time	,993	,171	2	,918	,994	1,000

Mauchly's test of sphericity tests the null hypothesis that the variances of the differences are equal.

The sphericity assumption is required for all univariate main effects and interaction tests.[33] Given Mauchly's test is impacted by non-normality and by sample size, it is not highly recommended when evaluating whether the sphericity condition has been met.

A Greenhouse-Geisser epsilon (ϵ) value < .75, suggests using the Greenhouse-Geisser adjustment with the univariate test of mean differences, whereas a value falling between .75 and 1 suggests the use of the Huynh-Feldt adjustment with the univariate tests. [(ϵ) = 1 is consistent with sphericity].

The sphericity assumed test was determined not to have been violated.

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Table 4: Output from two-way ANOVA (Test of Within-Subject Effects)

Source	Sum of squares	Degrees of freedom squares	Mean squares	F ratio	P-value	Partial Eta Squared	Noncent, Parameter	Observed Power
time	,648	2	,324	13,731	<,001	,337	27,462	,997
time* tx.group	,247	4	,062	2,616	,045	,162	10,463	,695
Error	1,274	54	0,24					

The main effect of time on pH values is statistically significant, sphericity assumed F (2, 54) = 13.73, p<,001. This effect was qualified by a significant time x tx. group interaction effect, sphericity assumed.

F (4, 54) = 2,616, p <,05.

Table 5: Output from two-way ANOVA (Test of Within-Subject Contrast)

Source	Time	Sum of squares	Degrees of freedom squares	Mean squares	F ratio	P-value	Partial Eta Squared	Noncent, Parameter	Observed Power
Time	Linear	,203	1	,203	8,619	,007	,242	8,619	,808
	Quadratic	,445	1	,445	18,824	<,001	,411	18,824	,987
Time* tx.group	Linear	,070	2	,035	1,476	,246	,099	2,952	,287
	Quadratic	,177	2	,089	3,751	,037	,217	7,503	,635
Error	Linear	,636	27	,024					
	Quadratic	,638	27	,024					

Although the test of the linear component of the trend is significant (p <,05), the higher-order quadratic component was also significant [F (1,27) = 18,824, p<,001]. This suggests that across groups, the mean level of pH exhibited a quadratic trend over the three measurement occasions.

Despite the fact that the test of the interaction between the linear component of the trend and treatment group is not significant, the interaction between the treatment group and the higher-order quadratic component was significant [F (2,27) = 3,751, p <,05.].

Table 6: Output from two-way ANOVA (Levene's Test of Equality of Error Variances)

	Levene Statistic	df1	df2	Significance
pH at Time 1 (6 hours) based on means	4,639	2	27	,019
pH at Time 2 (24 hours) based on means	4,893	2	27	,015
pH at Time 3 (48 hours) based on means	,360	2	27	,701

The Levene's test results involve tests of differences in variances at each time point, an assumption of the univariate ANOVA. It turns out that the standard Levene's test based on means are significant for Time 1 and Time 2. Nevertheless, a violation of this assumption is less of an issue with equivalent sample sizes.

Table 7: Output from two-way ANOVA (Tests of Between-Subject Effects)

Source	Sum of squares	Degrees of freedom squares	Mean squares	F ratio	P-value	Partial Eta Squared	Noncent, Parameter	Observed Power
Intercept	4575,606	1	4575,606	228898,999	<,001	1,000	228898,999	1,000
tx.group	,341	2	,170	8,528	,001	,387	17,056	,947
Error	,540	27	,020					

The Tests of Between-Subjects Effects is a test of the main effect of the grouping variable on pH values on the repeated measure averaged over time. The result presented here is simply a test of group differences on the average of pH values (i.e. those values averaged over time for each Candida broth culture.

The main effect of treatment group on the average pH values across time is statistically significant, F (2,27) = 8,528, p <,05. Based on estimated marginal means. *. The mean difference is significant at the ,05 level. b. Adjustment for multiple comparisons: Bonferroni

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Table 8: Output from two-way ANOVA (Estimated Marginal Means)

Treatment group	Mean	Std.Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Corega cream	7,156	,026	7,103	7,209
Corega powder	7,189	,026	7,136	7,242
Control	7,045	,026	6,992	7,098

Corega powder had the highest mean pH followed by Corega cream.

Table 9: Output from two-way ANOVA [Pairwise comparison on the average pH (averaged over time) for each treatment group]

(I) Treatment group	(J) Treatment group	Mean Difference (I –J)	Std.Error	Sig.[b]	95% Confidence Interval	
					Lower Bound	Upper Bound
Corega cream	Corega powder	-,033	,037	1,000	-,127	,060
	Control	,111*	,037	,016	,017	,204
Corega powder	Corega cream	,033	,037	1,000	-,060	,127
	Control	,144*	,037	,002	,051	,237
Control	Corega cream	,111*	,037	,016	-,204	-,017
	Corega powder	-,144*	,037	,002	-,237	-,051

Based on estimated marginal means.*. The mean difference is significant at the ,05 level. b. Adjustment for multiple comparisons: Bonferroni

The pairwise differences between Corega cream and Control as well as between Corega powder and Control were significant p <,05. The pairwise difference between Corega cream and Corega powder was not significant p >,05.

Table 10: Output from two-way ANOVA (Estimated Marginal Means)

Time	Mean	Std.Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	7,022	,028	6,964	7,080
2	7,230	,028	7,174	7,285
3	7,139	,028	7,084	7,193

The mean pH was highest during time 2 and intermediate during time 3.

Table 11: Output from two-way ANOVA [Pairwise comparison on the average pH irrespective of treatment group]

(I) Time	(J) Time	Mean Difference (I –J)	Std.Error	Sig.[b]	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-,207*	,041	<,001	-,312	-,103
	3	-,116*	,041	,020	-,217	-,015
2	1	,207*	,041	<,001	,103	,312
	3	,091	,041	,074	-,007	,189
3	1	,116*	,041	,020	,015	,217
	2	-,091	,041	,074	-,189	,007

The pairwise differences between time 1 and time 2 and between time 1 and time 3 were significant p <,05. The pairwise difference between time 2 and time 3 was not significant p >,05.

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Discussion

The present study was designed to determine whether there were significant mean differences in pH values over three measurement occasions from *C. albicans* cultures grown in the presence of acrylic resin plates treated with two forms of Corega, as well as whether there were group differences in terms of how the pH means varied over time.

Candida colony count

The current study found that colonies in the majority of plates plated with the 10^2 dilution were not in the countable range. This finding was unexpected and suggests serial dilution problems. It was surprising considering that a wide range of dilutions (10^5 – 10^3) were successfully plated. It is difficult to explain this result, but it might be related to air contaminants. Reynolds (2005) asserts that air contaminants can contribute significantly to a really low count.[34]

The most interesting finding was that in contrast to plates from the control group, plates from both the Corega cream and powder groups had no colonies in the countable range (25-250) during time 2. This result must be interpreted with caution. It suggests that both Corega cream and powder inhibit the growth of *C. albicans* albicans in contradiction to the previous research by Sampaia-Maio and colleagues (2012) which showed that among the different forms of Corega only the cream inhibits the growth of *C. albicans*.[8]

pH values

On the question of the main effects of time and treatment group on pH values, this study found significant mean differences in pH values over three measurement occasions (Table 4) as well as group differences in terms of how the means varied over time (Table 7) However, the results of this study showed a significant time x treatment group interaction. This indicates that, overall, the effect of time depended on the level of treatment group. In other words, the effect of time was different for different levels of treatment group. This means that interpretation of the main effects (time and treatment group) is incomplete or misleading.

The most interesting finding was that the pairwise differences in mean pH between Corega cream and Control as well as between Corega powder and Control were significant p <,05. This result has not previously been described. These findings suggest that both Corega cream and Corega powder raise the pH of the growth media. This action would inhibit the growth of *C. albicans* which prefers an acidic environment.[35]

Another important finding was that the pairwise difference in mean pH between Corega cream and Corega powder was not significant p >,05. This means that although Corega powder raises the pH of the growth media marginally higher (7,189 vs 7.156) than Corega cream the difference was not statistically significant.

Limitations of the study

Unanticipated number of plates with statistically insignificant number of colonies.

The Simple effects test was not performed to obtain more focused, specific information on where differences are in the interaction effect.

CONCLUSION

Both forms of the denture adhesive tested inhibited the growth of *C. albicans.*

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