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Research Article

The Effect of Residual Food Stain on Candida Albicans Colonisation of Denture Acrylics

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ABSTRACT

Objectives: In the UK, 19% of adults wear dentures. Failure to keep a denture clean can lead to staining from foods, along with subsequent colonisation of the denture and associated mucosa by microorganisms, particularly *Candida albicans*. This colonisation can potentially lead to chronic erythematous candidosis and other oral infections. This study investigated the association between staining of denture acrylics by different food types and subsequent *C. albicans* colonisation.

Materials and Methods: Chemically polymerised acrylic specimens were produced and stained for 14 days with six different combinations of food stains. The level of acrylic staining was determined spectrophotometrically. Specimens were then incubated in Sabouraud-dextrose broth (SAB) or SAB inoculated with *Candida albicans*. Confocal laser scanning microscopy coupled with propidium iodide staining of *C. albicans* was used to determine the extent of *C. albicans* colonisation to these acrylics. Results analysed descriptively and by one-way analysis of variance (ANOVA), one sample student t-test, and Dunnett's test.

Results: Acrylics in Group 4 (stained with spices, tomato puree, acai berry juice and sunflower oil) exhibited highest staining but had low *C. albicans* colonisation. Highest *C. albicans* colonisation occurred with Group 5 (sunflower oil) stained acrylics. The unstained control acrylic group had lowest colonisation.

Conclusion: This study demonstrated that staining acrylics with certain foods promoted *C. albicans* colonisation, but this was not associated with level of visual staining. Further research is required to determine the precise mechanism(s) by which residual food stains promote candidal colonisation on denture acrylics. This knowledge may then be used by dental professionals to advise patients on improving denture hygiene to improve not only denture aesthetics but also minimise *Candida* biofilms.

Introduction

Dentures are removable prosthetic appliances used to restore both function and aesthetics once a person has lost some or all of their natural teeth. The 2009 Adult Dental Health survey outlined the changing trend of tooth loss since the initial survey in 1968, *i.e.* levels of edentulousness had fallen in recent times and there was a relationship between increasing age and tooth loss [1]. Approximately 20% of the UK population wear partial dentures (13%) or complete dentures (6%). Furthermore, 70% of those aged 75 years or over, wear dentures and this is indicative of the great demand for the provision of dentures as life expectancy increases [2].

The most commonly isolated microorganism, which can pathologically grow on the surface of dentures, is *Candida albicans*. *Candida albicans* is part of the commensal oral microflora in many individuals, but its levels can rapidly elevate in denture wearers. In the presence of a denture, which can lower oxygen and saliva flow over the underlying mucosa, *C. albicans* can rapidly colonise and become pathogenic. If left untreated, this can lead to the development of chronic erythematous candidosis or denture-induced stomatitis [3, 4]. Similarly, if a patient

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wearing a partial denture does not maintain good oral hygiene, increased plaque and bacterial levels can lead to plaque-mediated diseases such as dental caries (on the remaining teeth) or periodontal diseases [5]. There are many foods, which can stain both the acrylic base and the teeth of dentures. While the colour of denture teeth may be the most important aspect to patients, staining of the acrylic denture base can also lead to aesthetic concerns.

As a society, the UK is becoming increasingly multi-cultural, with the 2016 census (Office of National Statistics) indicating that 39% of the population was born outside of the UK. Increased diversity has resulted in changes in dietary habits, with spices such as turmeric, paprika and cumin along with tomato puree, becoming increasingly popular in recent years. While the staining of denture acrylics by drinks such as coffee, tea and red wine has been widely studied, this current research focused on foods including spices, tomato puree, acai berry juice and sunflower oil [6, 7]. The research aimed to ascertain which food groups caused highest level of acrylic staining and whether this food staining resulted in enhanced *C. albicans* colonisation of the acrylic surface. The null hypothesis was that staining by certain food types would not affect subsequent colonisation of *C. albicans* on denture acrylics.

Materials and Methods

All the acrylics were prepared in the same manner and at the same time. Selection of acrylic coupons for test and controls was random and from the pool of prepared materials. Some previous study has suggested that staining can affect surface roughness, which could influence microbial adherence. This is an area that we are currently exploring further. Specimens of denture acrylic were fabricated using chemically polymerised polymethyl-methacrylate (PMMA). This was prepared by mixing PMMA polymer powder and methyl-methacrylate monomer liquid in a 2:1 ratio. The product of the denture base resin was 'Oracryl' (Bracon Limited, East Sussex, UK).

All specimens were uniformly prepared for the test groups, and once polymerised, the specimens were removed from the matrix and cut to the required dimensions [30mm x 20mm x 3mm] without adjustment to the surface finish. Suitable specimens were then selected following exclusion of those with any noticeable porosities or air blows. The coupons were used *in lieu* of 'real dentures' because their flat, abraded surface provided an adequate size area for uniform bacterial plaque growth, staining, and allowed for simple and reproducible quantitative analysis. Excess debris was removed from the PMMA coupons by soaking at 50ºC in a 0.2% aqueous detergent solution for 1 hour. The specimens were rinsed under running hot water, dipped in methanol, and allowed to air dry on paper towels. Specimens were also autoclaved for 15 minutes at 121ºC at 15 psi and allowed to cool to room temperature.

Specimens (n=4) of acrylic resin were assigned to a test staining group (Table 1). Group 1 stains consisted of turmeric, cumin, paprika and sunflower oil; Group 2: Tomato puree and sunflower oil; Group 3: Turmeric, cumin, paprika, tomato puree and sunflower oil; Group 4: Turmeric, cumin, paprika, tomato puree, acai berry juice and sunflower oil; Group 5: Sunflower oil; and Group 6: distilled water. Pre-staining colour readings taken using a CR-400 Chroma-Meter or Spectrophotometer CM-700d. Test staining solutions were prepared by adding 1g of components to 50ml of sunflower oil on a heated stirrer. Acrylic specimens were immersed in the prepared solutions for 14 days at 37°C and then dried. Control specimens were submerged in 2ml of Sabouraud-dextrose broth (SAB). The colour change between the pre and post staining was measured by calculating the Delta E values indicative of the colour change of three coordinates: L* (lightness), a* (red and green) and b* (yellow and blue). The coordinates were then used to locate the colour of the specimen in the CIE, L*a*b* colour space, which is a three-dimensional model describing all the colours visible to the human eye [8].

Table 1: The food constituents of each acrylic staining group along with a control.

Group number	Constituents
1	Turmeric, cumin, paprika and sunflower oil
2	Tomato puree and sunflower oil
3	Turmeric, cumin, paprika, tomato puree and sunflower oil
4	Turmeric, cumin, paprika, tomato puree, acai berry juice and sunflower oil
5	Sunflower oil
6 (Control)	Distilled water

Test and control acrylic specimens were wiped with alcohol, to remove surface debris, prior to incubating in 1ml of a standardised (optical density of 1.0 at a wavelength of 540nm) inoculum of *Candida albicans* ATCC 90028 for 72 h at 37°C. After incubation, acrylic specimens were fixed in 10% formal saline and then stained with propidium iodide. Confocal laser scanning microscopy (CLSM) was then used to assess the number of colonising *Candida*. Quantification of *C. albicans* (yeast and hyphal forms) was based on the method described in the ImageJ user guide (Link) [9]. Images were adjusted with regards to their threshold intensity, and processed through the "analyse particles" tool, using the "outlines" function. Yeast were identified according to size (0.1-100 μm^2) and circular shape (0.5-1.0 diameter) and hyphae by changing the size (0-infinity μm^2) and shape (0.0-0.3 μm^2) parameters. Scanning electron microscopy (SEM; see supplementary figures) allowed further assessment of surface characteristics of the acrylic.

Statistical analysis, including one-way analysis of variance (ANOVA) and Dunnett's test, was used to determine statistical differences in the pre and post staining colour changes as well as *C. albicans* colonisation. A P value of < 0.05 was deemed statistically significant. All statistical analyses were carried out using GraphPad Instat version 3.10 for Windows [10]. Boxplots and barplots were created using R scripts.

Results

Table 2 presents the four colour readings taken of each set of specimens, prior to and after immersion in the staining solutions highlighting differences between groups. The colour change for each coordinate ΔL^*, Δa^* and Δb^* was measured, followed by a calculation of Delta E (ΔE). Figure 1 presents the results and shows the colour change values of stained acrylics using the Delta E calculation (implemented in R scripts). Staining groups 1 and 4 showed the most significant change in staining compared with other groups and controls.

Table 2: Colour analysis of stained acrylics (using the Delta E calculation) prior to and after staining.

		Prior				**After**			
Specimen number		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Group 1	L	54.0	52.7	53.8	54.8	58.4	58.0	57.6	57.4
	A	17.3	17.0	17.1	16.9	9.6	10.8	10.5	9.9
	B	4.2	3.9	4.2	4.1	19.3	13.9	18.6	24.5
Group 2	L	55.6	55.7	55.2	55.5	52.0	51.4	50.6	50.7
	A	16.8	16.6	15.8	17.3	18.6	18.0	18.0	18.1
	B	3.9	3.5	2.3	3.8	3.2	2.0	1.1	1.6
Group 3	L	51.2	51.4	51.7	51.3	52.5	53.4	52.5	52.1
	A	17.3	17.5	17.7	17.9	16.8	15.5	16.6	17.0
	B	1.3	1.7	2.5	2.0	8.1	8.4	6.9	7.0
Group 4	L	53.6	53.1	53.3	53.2	52.9	53.3	52.1	52.8
	A	16.5	16.4	17	16.7	11.3	11.8	11.4	11.3
	B	1.4	1.3	1.3	1.7	29.2	27.6	30.1	29.6
Group 5	L	59.4	59.1	59.7	59.1	52.9	52.6	52.1	53.9
	A	15.9	15.7	15.6	16.3	16.9	18.3	16.8	17.1
	B	4.7	4.4	4.0	4.3	3.8	4.7	3.5	4.4
Group 6	L	52.3	52	52.2	52.4	51.6	51.2	51.4	51.1
	A	18.1	17.3	17.5	18.1	17.4	18.4	18.1	18.3
	B	3.9	3.6	3.4	3.5	-	-	-	-

Table 3: Descriptive statistical analysis, Dunnett's analysis and the differences in *Candida albicans* colonisation for each acrylic stained group.

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
Descriptive statistical analysis						
Mean	17.1	4.8	5.9	28.1	6.6	1.3
Std.	3.6	0.5	1.3	1.1	1.0	0.1
Sample size	4	4	4	4	4	4
Std. error of mean	1.8	0.2	0.6	0.5	0.5	0.05
Lower 95% conf. limit	11.3	3.9	3.8	26.4	5.0	1.1
Upper 95% conf. limit	22.9	5.7	8.0	29.9	8.2	1.4
Minimum	12.9	4.0	4.6	26.7	5.2	1.2
Median (50th percentile)	16.9	5.0	6.0	28.3	6.8	1.3
Maximum	21.7	5.3	7.2	29.3	7.7	1.4
Dunnett's analysis						
Mean difference	-15.9	-3.5	-4.6	-26.8	-26.8	NA
q value	13.0	2.9	3.8	22.1	4.4	NA
P value	P<0.01	P<0.05	P<0.01	P<0.01	P<0.01	NA
Differences in *Candida albicans* colonisation for each acrylic stained group.						
Number	1837	837.6	411.6	379.8	2882.6	209

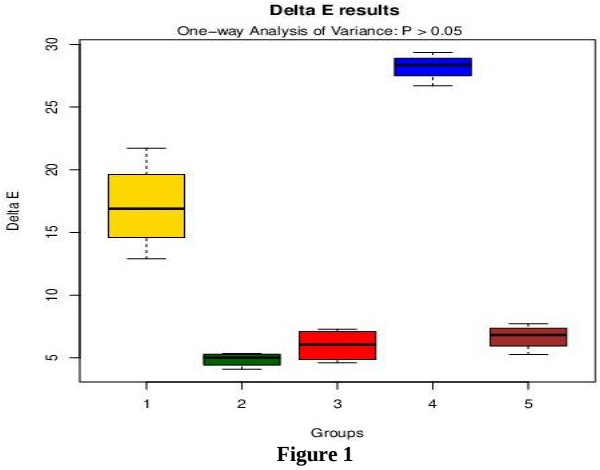

Figure 1

Of the staining groups, highest acrylic staining occurred with Group 4, with an average Delta E value of 28.194, and lowest was with Group 2 (Delta E = 4.857). Overall, the control group showed the lowest staining, with a Delta E value of 1.314. ANOVA showed the p value to be < 0.0001, which indicated that the variation among the means of the groups was significantly greater than would be expected by chance. Dunnett's test compared the test groups' results to that of the control group with a q value of greater than 2.760 highlighting the p value was less than 0.05. Therefore, the colour change difference between all of the staining groups and the control group was statistically significant (Table 3).

The quantity of *C. albicans* colonisation as measured by imaging was determined, and the mean colonization level subsequently calculated. Images obtained were analysed using ImageJ to quantify surface area coverage of the fluorescent *Candida*. Using one sample student t-test,

there was a significant difference ($P<0.05$) between the six groups in terms of *Candida* colonisation with a confidence interval of 95% [-15.08, 2201.14]. The overall mean for all groups was 1093.03 with a standard deviation of 1055.91.

Acrylic stained in sunflower oil (Group 5) had the highest mean number of *C. albicans* (2882.6 per unit area), whilst the control group had lower colonisation (*C. albicans* =209). Group 4, the most stained specimen, had the least amount of *C. albicans* out of all the test groups. This indicated that visually increased staining of denture acrylics did not necessarily lead to increased colonisation of *C. albicans* on the acrylic surface. Highest *C. albicans* colonisation was associated with acrylics stained only with sunflower oil (Group 5).

Discussion

The study was undertaken to investigate which of the tested foods resulted in highest staining of denture acrylics, and also whether the effect of this staining would impact on colonisation of the acrylic surface by *C. albicans*. The significant differences between the staining groups showed that the chosen foods did indeed stain denture acrylic. Johnston and Kao stated that a delta E value of 3.7 or less was acceptable to denture users with regards to staining [11]. In this study, the only group that did not have a clinically visible difference in colour was the control (Delta E value = 1.31). The staining groups were chosen to represent foods that are becoming increasingly popular in British cuisine with the growing multicultural nature of society. While dentists have long known about the staining effects of drinks such as red wine, coffee and tea, it is important that other aspects of the diet are explored as food habits change. As spices are primarily used in conjunction with one another and rarely used alone, the study used different combinations of commonly used spices.

Acai berry juice along with turmeric, cumin, paprika, tomato puree and sunflower oil produced the greatest colour change ($\Delta E = 28.19$) in the denture acrylics. It would, therefore, be of interest in the future to investigate the staining of acai berry juice alone on dentures. SAB medium was chosen as a negative control as it was comparatively colourless, and as expected, exhibited minimal acrylic staining ($\Delta E = 1.31$). The minor colour change observed could possibly be as a result of slight water absorption by the acrylic during the 14 days of being submerged. Specimens were submerged in the staining solutions for 14 days. This was done to allow for sufficient staining to occur and under the assumption that the chosen foods would be regularly consumed in patients that would result in similar degrees of denture staining. An unclean denture that was allowed to accrue such staining is also likely to acquire a salivary pellicle and plaque layers. This would further alter the surface of the acrylic and thus, potentially affect microbial growth.

Candida albicans successfully colonised all test acrylic specimens. The control had the lowest mean number of *C. albicans* (n=209), which would suggest that staining of denture acrylics did indeed increase colonisation with respect to the five groups tested. However, there was no correlation between the level of staining and colonisation by *C. albicans*. The most stained specimens (Group 4) had the least number of *C. albicans* (n=379.8), while highest colonisation occurred in Group 5 (n=2882.6), which had been submerged in sunflower oil. It is possible therefore that different food components were being adsorbed into the acrylic, and these served as receptors for attachment of *Candida*. Potentially food components could also have influenced subsequent growth over the following 72 h in a positive or negative manner. An area for further research would be to investigate the overall effect of a stained, unclean denture in the oral environment and the subsequent growth of *C. albicans*. Such an approach could be expanded to assess the merits of various denture cleaners to remove stains and thus their impact on denture colonisation by microorganisms.

Conclusion

This study demonstrated differences between various food groups in staining denture acrylic. Furthermore, in response to a lack of knowledge on the effect of residual food stains on microbial colonisation of denture acrylics, this study showed that certain foods promote *C. albicans* colonisation of these surfaces. A further aspect of this research would be to undertake these investigations in the presence of saliva or in the oral cavity of denture wearers, where diet and the level of existing staining on dentures could also be recorded. Should a definitive link be identified *i.e.* the type of stain and *C. albicans* adherence/growth, this would be useful information for both dentists and patients. Patients could be given more detailed dietary and denture hygiene advice during the fitting of dentures. This would be beneficial from both a denture aesthetics perspective and also from the view of reducing *C. albicans* colonisation.

This in turn may reduce the incidence of inflammatory conditions such as denture-induced stomatitis, which can be both harmful and uncomfortable for patients. With around 1 in 5 adults in the UK wearing dentures, further research in denture staining and microbial colonisation of denture acrylics would increase the knowledge base, improve diagnostics and treatment that would be beneficial for both dental professionals and patients.

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