

RESEARCH ARTICLE

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Chemical, mechanical, and biological effects of repeated chlorhexidine disinfection on acrylic and polyamide denture base materials: A six-month in vitro study

Chlorhexidine effects on denture polymers

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Abstract

Objective: This study aimed to evaluate the chemical stability, leachate release, surface properties, mechanical properties, water sorption, solubility and cytocompatibility of polymethyl methacrylate (PMMA) and polyamide denture base materials after 6 months of repeated disinfection with chlorhexidine.

Methods: Heat-polymerised PMMA specimens and injection-moulded polyamide specimens were subjected to distilled water, 0.12% chlorhexidine and 2% chlorhexidine. The immersion time for each day was simulated as 10 minutes, and the rinsing time for each day was simulated as 60 seconds for six months.

Results: Chlorhexidine caused concentration-dependent changes in both materials. Following this simulated 6-month period, 0.12% chlorhexidine produced an increase in surface roughness ($0.31 \pm 0.05 \mu\text{m}$ in PMMA and $0.34 \pm 0.06 \mu\text{m}$ in polyamide), and a decrease in flexural strength ($83.8 \pm 6.4 \text{ MPa}$ in PMMA and $69.8 \pm 5.5 \text{ MPa}$ in polyamide). For PMMA, $1.74 \pm 0.28 \mu\text{g/mL}$ of methyl methacrylate was released, while for polyamide, $0.91 \pm 0.19 \mu\text{g/mL}$ of caprolactam-related compounds was released. The viability of the fibroblasts decreased to $78.6 \pm 6.2\%$ and $84.9 \pm 5.8\%$, respectively. Exposure to 2% chlorhexidine resulted in higher deterioration, with the PMMA cell viability reduced to $66.9 \pm 6.4\%$.

Conclusion: Repeated chlorhexidine disinfection produced concentration-dependent changes in both PMMA and polyamide denture base materials. The 2% chlorhexidine group showed the greatest chemical, mechanical, surface, and biological deterioration. PMMA released more methyl methacrylate and caused greater fibroblast viability reduction, whereas polyamide showed higher water sorption and surface roughness. Routine daily immersion in 2% chlorhexidine should therefore be avoided or used with caution.

Keywords: Chlorhexidine, Denture bases, Polymethyl methacrylate, Polyamide, Cytocompatibility.

Plain English Summary

In this laboratory study, the authors hypothesised that the repeated use of chlorhexidine for six months would not affect acrylic or polyamide denture bases. A daily 10-minute immersion followed by 60 seconds of rinsing was simulated for six months. Chemical, surface, mechanical and biological changes were observed in both types of materials following the 6 months of exposure. Acrylic showed a higher amount of residual monomer and a higher decrease in the viability of gingival fibroblasts than polyamide. Polyamide

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absorbed a higher amount of water and had a higher surface roughness. The greatest changes were obtained with 2% chlorhexidine. Thus, high-concentration chlorhexidine solutions should not be routinely used for daily denture immersion.

Introduction

Removable dentures continue to be a crucial type of treatment in cases where a patient has missing teeth, either all or some, and is not a candidate for implant treatment due to anatomical, medical, or financial reasons. The durability of these prostheses relies on the fit and design of the prosthesis, as well as on the capacity of the denture base material to resist repeated exposure to saliva, food particles, temperature fluctuations, microbial films and cleaning solutions. These factors can gradually impact the surface quality, mechanical properties, and biological safety of the product (1, 2).

Polymethyl methacrylate (PMMA) is the most commonly used denture base material, due to its satisfactory esthetic qualities, low cost, ease of processing and repair and relining. Incomplete polymerisation, however, can cause the presence of low-molecular weight components such as residual methyl methacrylate in the resin. They can be released over time of water storage, or repeated chemical exposure, which may impact material strength and compatibility with oral tissues (1, 3, 4). Flexible partial dentures and metal-free clasp designs are often fabricated of thermoplastic polyamide. The flexibility also makes it impact-resistant, with a possible improvement in comfort and a decreased likelihood of abrupt fracture. However, polyamide materials are generally less easily polished and may be more water-absorbing due to the amide groups present in the polymer structure. Greater water uptake can affect the dimensional stability, surface roughness, hardness, and the ability of soluble compounds to be released (2, 5, 6).

Adequate denture hygiene is necessary to control microbial accumulation and reduce the risk of denture stomatitis. Mechanical brushing remains important, but it may be less effective on irregular surfaces and in areas that are difficult to access. Chlorhexidine is therefore frequently used as an additional chemical disinfectant because of its

broad antimicrobial activity against oral bacteria and *Candida* species. However, repeated exposure to chlorhexidine may also interact with denture polymers and gradually alter their physical and chemical properties (7, 8, 9, 10).

Many previous studies have been conducted to determine the short-term changes in colour, roughness, hardness or antimicrobial activity. Little data is available about the long-term effects of chlorhexidine on the polymer structure, release of leachates, mechanical properties and cytocompatibility. The effects of repeated exposure to 0.12% and 2% chlorhexidine on PMMA and polyamide denture base materials over a simulated six-month period were therefore evaluated in this study. Chemical changes, release of compounds, surface roughness, microhardness, flexural strength, water sorption, solubility and gingival fibroblast viability have been measured. The idea was that the type of material, chlorhexidine concentration and their interaction would have a major effect on the outcomes measured.

Materials and Methods

Study design and sample size

The effect of repeated chlorhexidine disinfection was tested in vitro on polymethyl methacrylate (PMMA) denture base materials heat polymerised and on denture base materials injection moulded. Distilled water, 0.12% chlorhexidine gluconate, or 2% chlorhexidine gluconate were applied to specimens for 180 daily cycles (around 6 months of use).

Sample size was calculated for a 2-way factorial design using G*Power version 3.1.9.7, assuming an effect size of 0.35, 80% statistical power and a two-sided 5% significance level. For each outcome, there were 8 independent specimens allocated for each material–solution subgroup (2 materials × 3 immersion solutions × 8 specimens). To prevent repeated-testing bias, separate sets of specimens were used for destructive or method-incompatible tests (Table 1).

Table 1: Experimental groups and sample allocation

Material	Distilled water	0.12% CHX	2% CHX	Total per outcome
PMMA	n=8	n=8	n=8	n=24
Polyamide	n=8	n=8	n=8	n=24
Total	n=16	n=16	n=16	n=48

Separate specimen sets were prepared for FTIR/HPLC, surface testing, flexural strength, water sorption/solubility, and cytocompatibility analyses. CHX, chlorhexidine; PMMA, polymethyl methacrylate

Materials and specimen preparation

For this study, heat-polymerised polymethyl methacrylate resin (Meliodent Heat Cure, Kulzer GmbH, Hanau, Germany) and injection-moulded thermoplastic polyamide resin (Valplast, Valplast International Corporation, Westbury, NY, USA) were employed. The immersion solutions were distilled water, an alcohol-free 0.12% chlorhexidine mouthwash (KIN Gingival 0.12, Laboratorios KIN S.A., Barcelona, Spain) and newly prepared 2% chlorhexidine digluconate. The 2% chlorhexidine solution was prepared by diluting 20% chlorhexidine digluconate stock solution (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) with sterile distilled water right before application.

The specimens of PMMA were prepared by the method of packing the dough and polymerising it by applying a water-bath curing cycle at 74°C for 8 hours and at 100°C for 1 hour, with a 2.5:1 powder-to-liquid ratio (by weight). The specimens were bench cooled to room temperature before deflasking. Samples of polyamide were injection-moulded following the manufacturer's instructions.

Instrumentation

ATR-FTIR spectra were obtained with the ALPHA II FTIR spectrometer, which is equipped with the attenuated total reflectance accessory (ATR) (Bruker Optics GmbH & Co. KG, Ettlingen, Germany). HPLC analysis was carried out with a Shimadzu LC-20AT Prominence HPLC system with UV detector (Shimadzu Corporation, Kyoto, Japan) on a C18 analytical column (ZORBAX Eclipse XDB-C18 250 × 4.6 mm, 5 µm; Agilent Technologies, Santa Clara, CA, USA). The surface roughness was quantified with a contact profilometer (SurfTest SJ-210, Mitutoyo Corporation, Kawasaki, Japan). Microhardness was measured by a micro-Vickers hardness tester (HVM-G31, Shimadzu Corporation, Kyoto, Japan) following the Vickers microhardness method. The flexural strength was determined with a three-point bending fixture and a universal testing machine (Instron 3345, Instron, Norwood, MA, USA). The absorbance was measured with a microplate reader (ELx800, BioTek Instruments Inc., Winooski, VT, USA).

Disinfection and ageing protocol

The specimen was put into 20 mL of its designated solution and left there for 10 minutes at 37 °C, followed by 60 seconds of rinsing with distilled water and stored in artificial saliva until the next cycle. The solutions were stirred at about 60 rpm. Artificial saliva had the following composition: 0.4 g/L sodium chloride, 0.4 g/L potassium chloride,

0.795 g/L calcium chloride dihydrate, 0.69 g/L sodium dihydrogen phosphate dihydrate, 0.005 g/L sodium sulphide nonahydrate, and 1.0 g/L urea. The pH was adjusted to 6.8 ± 0.1 , and it was replaced every 48 hours. A new solution of chlorhexidine was used for each cycle.

Fourier-transform infrared spectroscopy

Attenuated-total-reflectance Fourier-transform infrared spectroscopy (a.t.r.-FTIR) was used to assess chemical changes with 32 scans of 4 cm⁻¹ resolution in the spectral range between 4,000 and 500 cm⁻¹.

The carbonyl band of the esters (around 1720 cm⁻¹) and the region of stretching vibrations of C–O were evaluated for PMMA. The amide I and amide II region was examined in the case of polyamide. The spectra were baseline corrected and normalised. Stable internal reference bands were used to determine carbonyl and amide-related indices.

Specimens were aged, then rinsed and allowed to age for 24 hours in sterile phosphate-buffered saline at 37°C at a surface area of 3 cm² per volume of 1 mL. The extracts were filtered through 0.22-µm filters and split for chromatographic and cytocompatibility analysis.

We quantified methyl methacrylate released from PMMA and caprolactam released from polyamide using high-performance liquid chromatography (HPLC) with a C18 column of 250×4.6 mm, 5-µm particles.

Acetonitrile–water (60:40) at 1.0 mL/min, 25°C, and 210 nm was used for the analysis of methyl methacrylate. Water–methanol (85:15) was used for the analysis of caprolactam at 1.0 mL/min, 30°C, and 210 nm. The volume of the injection was 20 µL.

Methyl methacrylate standards were prepared in the range of 0.10–5.0 µg/mL, and caprolactam standards were prepared in the range of 0.05–2.5 µg/mL. Intra- and inter-day RSD were considered acceptable if < 5%, and a coefficient of determination > 0.995 and a recovery range between 90 and 110% were acceptable values. Peaks which could not be identified were recorded by retention time but not chemically identified without the aid of mass-spectrometric confirmation.

Surface roughness and microhardness

A contact profilometer was used to measure surface roughness. A cut-off length of 0.8 mm, an evaluation length of 4 mm and a stylus speed of 0.5 mm/s were used to record three traces for each specimen. The mean arithmetic roughness was reported in micrometres (Ra).

The microhardness was measured with Vickers microhardness testing under a load of 25 kg for 15 seconds. Five impressions were taken on each specimen, and the average of these impressions was used as the final value of hardness.

Flexural strength

The flexural strength was determined using a three-point bending fixture of the universal testing machine. Specimens were loaded at a rate of 5 mm/min across a 50 mm span until failure or permanent failure. Flexural strength (σ , MPa) was calculated as follows: $\sigma = 3FL/(2bd^2)$ in which F is the maximum load at failure (N), L is the support span (mm), b is the specimen width (mm), and d is the specimen thickness (mm).

Water sorption and solubility

Specimens were dried in a desiccator at 37°C until the initial weight was obtained. The mass after ageing was measured, and then the sample was redried to get the final constant mass. Water sorption (Wsp) and solubility (Wsl) were determined by the following equations: $Wsp = (m2 - m3)/V$, and $Wsl = (m1 - m3)/V$, where m1 is the mass of the initial conditioned sample, m2 is the mass after immersion, m3 is the mass after reconditioning, and V is specimen volume in mm³.

Exposure of cells to culture and extracts. Cell culture and extract exposure.

Human gingival fibroblasts were cultured at 37°C and 5% carbon dioxide in Dulbecco's modified Eagle medium containing 10% foetal bovine serum (FBS) and 1% penicillin–streptomycin (PS).

Cells from passages 4-8 were plated in 96-well plates at 1×10^4 cells/well and incubated for 24 hours to attach. The specimen extracts were then placed into the culture medium. Untreated medium was used as a negative control, whereas 0.1% Triton X-100 was used as a positive control. The experiments were repeated three times, and each condition was tested in triplicate.

MTT assay

MTT was then added at 0.5 mg/mL and incubated for 4 hr after 24 hrs of exposure. The formazan crystals obtained were dissolved in 100 μ L of dimethyl sulfoxide, and the absorbance was read at 570 nm using 630 nm as a reference.

Extracts were tested at 50% dilution in culture medium and undiluted. Cells were serum-starved for 4 hours before exposure to the extract. Triton X-100 0.1% was used as a positive control and achieved a significant decrease in viability, thus proving the validity of the assay.

Blinding and reliability

When possible, specimens were coded to hide group allocation for measurement purposes. Twenty per cent of specimens were remeasured, and the intraclass correlation coefficient (ICC) was accepted as at least 0.80. Duplicate analysis was repeated if a difference of more than 5% was found.

Statistical analysis

IBM SPSS Statistics version 29.0 was used to analyse data. The normality and homogeneity of variance tests were used to evaluate normality and uniformity of variance, respectively. Data are shown as mean $\pm$ SD.

Two-way factorial analysis of variance was used to analyse each outcome, with material type (PMMA or polyamide) and immersion solution (distilled water, 0.12% chlorhexidine or 2% chlorhexidine) as fixed factors. Simple-effects analysis and Tukey-adjusted pairwise comparisons were used when a significant interaction was found. The family-wise significance level was determined as $p < 0.05$.

Partial eta squared (partial η^2) was reported for effect sizes and interpreted as small (0.01), moderate (0.06), or large (0.14). Pearson's correlation coefficient was used to explore the correlation between leachate concentration and selected physical, mechanical and biological results, where the assumptions were met; otherwise, Spearman's rank correlation was used. If applicable, 95% confidence intervals were reported.

Results

All specimens were immersed through the cycles with no fractures, deformation or loss of material. Surface dullness was seen in the 2% groups after long-term exposure to chlorhexidine. The PMMA showed lower surface roughness and higher microhardness and flexural strength as compared to polyamide at baseline.

Chemical changes and leachate release

Concentration-dependent changes of the FTIR indices were obtained for chlorhexidine. After the simulated period, the PMMA carbonyl index decreased from 1.00 ± 0.04 at baseline to 0.86 ± 0.05 with 0.12% chlorhexidine and 0.79 ± 0.06 with 2% chlorhexidine. The polyamide amide index decreased from 1.00 ± 0.05 to 0.89 ± 0.06 and 0.83 ± 0.07 , respectively.

There was a significant difference in leachate release between chlorhexidine concentrations and between the types of materials ($p < 0.001$). PMMA released more MMA than the amount of

caprolactam released from polyamide. Although prolonged exposure to 2% chlorhexidine revealed some small unidentified peaks on the

chromatogram, they could not be identified chemically (Table 2).

Table 2: FTIR indices and leachate concentrations

Material	Immersion solution	Relative FTIR index	Leachate concentration (µg/mL)
PMMA	Distilled water	0.93±0.05	0.68±0.16
PMMA	0.12% chlorhexidine	0.86±0.05	1.74±0.28
PMMA	2% chlorhexidine	0.79±0.06	2.31±0.35
Polyamide	Distilled water	0.95±0.05	0.42±0.11
Polyamide	0.12% chlorhexidine	0.89±0.06	0.91±0.19
Polyamide	2% chlorhexidine	0.83±0.07	1.18±0.24

Data are expressed as the mean ± standard deviation. The FTIR index represents the carbonyl index for PMMA and the amide index for polyamide. Leachates are, in this case: methyl methacrylate for PMMA and caprolactam for polyamide.

Surface and mechanical properties.

The surface roughness was found to increase with increasing chlorhexidine concentration, and the microhardness and flexural strength values were

found to be lower for higher concentrations of chlorhexidine. The 2% chlorhexidine solution caused the most damage to both materials after 6 months. The surface roughness, microhardness, and flexural strength were significantly affected by both material type and immersion solution ($p < 0.001$).

PMMA specimens generally fractured suddenly during three-point bending, whereas polyamide specimens showed marked bending before failure (Table 3).

Table 3: Surface and mechanical properties of PMMA and polyamide after six months of immersion

Material	Immersion solution	Surface roughness (µm)	Vickers hardness	Flexural strength (MPa)
PMMA	Distilled water	0.22±0.04	19.6±1.3	89.7±6.6
PMMA	0.12% chlorhexidine	0.31±0.05	18.1±1.3	83.8±6.4
PMMA	2% chlorhexidine	0.38±0.06	16.7±1.4	77.9±6.1
Polyamide	Distilled water	0.27±0.05	11.4±1.0	73.8±5.7
Polyamide	0.12% chlorhexidine	0.34±0.06	10.5±1.0	69.8±5.5
Polyamide	2% chlorhexidine	0.42±0.07	9.6±0.9	64.7±5.3

Data are reported as mean ± SD.

Water sorption, solubility and cytocompatibility.

Polyamide had higher water sorption and higher solubility than PMMA. The higher the concentration of chlorhexidine, the higher the outcomes: after 6 months in 2% chlorhexidine, the highest figure was obtained (Table 4).

The distilled-water groups maintained fibroblast viability >90%, as indicated by the extracts. The

fibroblast viability was decreased following chlorhexidine exposure, especially for the PMMA groups. The mean viability of only the PMMA group treated with 2% chlorhexidine for 6 months fell below the 70% cytotoxicity limit. Figure 1 shows the trend in fibroblast viability among the different groups.

Table 4: Water sorption and solubility, and fibroblast viability.

Material	Immersion solution	Water sorption (µg/mm ³)	Solubility (µg/mm ³)	Cell viability (%)
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PMMA	Distilled water	21.8±2.3	1.03±0.24	91.4±5.3
PMMA	0.12% chlorhexidine	24.6±2.5	1.47±0.27	78.6±6.2
PMMA	2% chlorhexidine	27.9±2.8	1.86±0.31	66.9±6.4
Polyamide	Distilled water	25.7±2.6	1.31±0.27	92.7±5.1
Polyamide	0.12% chlorhexidine	29.4±2.9	1.82±0.32	84.9±5.8
Polyamide	2% chlorhexidine	33.2±3.1	2.38±0.38	75.8±6.1

Values are presented as mean±standard deviation. Cell viability below 70% was interpreted as an in vitro cytotoxic response.

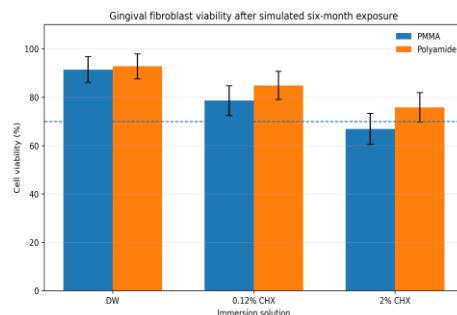


Figure 1: Gingival fibroblast viability following simulated six-month exposure of PMMA and polyamide specimens to distilled water, 0.12% chlorhexidine, and 2% chlorhexidine. Error bars represent standard deviations. The dashed line indicates the 70% in vitro cytotoxicity threshold.

Correlation analysis

The surface roughness of PMMA and polyamide had positive correlation with leachate concentration ($r=0.78$, $p<0.001$; $r=0.69$, $p<0.001$, respectively). Lower microhardness, flexural strength and fibroblast viability were related to higher concentrations of leachate. The highest correlation was found between PMMA cell viability and release of methyl methacrylate ($r=-0.81$, $p<0.001$).

The overall results showed that moderate changes were caused by the use of 0.12% chlorhexidine, whereas 2% chlorhexidine caused more chemical, mechanical and biological alterations after 6 months of exposure.

There was a significant immersion-solution effect for all tested outcomes (all $p<0.001$) and large effect sizes for most variables on the outcome of factorial analysis. The leachate concentration, surface roughness, microhardness, flexural strength, water sorption, solubility and cell viability were found to be significantly different depending on the type of material. Only for leachate concentration a significant material $\times$ solution interaction was found, and the solid matrix of PMMA and polyamide showed different behaviour with respect to the compounds released as a function of the different amounts of chlorhexidine.

Discussion

The present study has shown that six months of repeated chlorhexidine exposure resulted in chemical, surface and mechanical changes as well as biological changes in both PMMA and polyamide denture base materials. The extent of the changes was found to be primarily dependent on the concentration of chlorhexidine and the material type; thus, the null hypothesis was rejected. The 2% chlorhexidine caused more damage than the 0.12% chlorhexidine, suggesting that higher concentrations can be more harmful to denture polymers after multiple uses (11, 12, 13, 14, 15, 16, 17, 18).

FTIR results showed changes in the chemical composition of the surface of both polymers were observed after 6 months of repeated exposures. The decrease in the relative carbonyl index and the broadening of hydroxyl-associated regions in PMMA could be due to increased water uptake, local changes in the polymer environment, and/or early degradation. These changes in the spectra were paralleled by an increase in solubility, leachate emission and a decrease in hardness, thus suggesting progressive modification instead of mere surface deposition. However, the intensity of the peaks in FTIR is not necessarily indicative of bond breaking and must be interpreted in conjunction with the other results (1, 3, 4, 11).

Similar concentration-dependent changes in the amide regions were observed for polyamide. The presence of polar amide groups can cause it to interact with water and molecules in the immersion medium by hydrogen bonding, resulting in greater uptake of fluid and influence on polymer-chain organisation. This mechanism can be a possible reason for higher water sorption in polyamide. The present results do not, however, apply to all flexible denture materials as there are differences in the composition of the monomers, crystallinity, processing conditions, and additives (2, 5, 6).

A significant finding was that material-related compounds were released more after chlorhexidine exposure. The concentration of PMMA released increased with the concentration of chlorhexidine after 6 months of exposure. Chlorhexidine does not appear to produce significant amounts of methyl methacrylate. A more likely scenario is that the repeated penetration of the solution and polymer plasticisation resulted in the movement of any low molecular weight materials (such as residual monomer) in the resin. The polymerisation cycle, degree of conversion, storage conditions and water penetration are all factors affecting residual release of MMA (3, 4).

Polyamide had lower release of caprolactam than PMMA had of methyl methacrylate, which might contribute to the higher fibroblast viability measured in the polyamide groups. However, the chemical composition of thermoplastic denture materials is not always completely known and not all polyamides are derived from the same monomer systems. If these small unidentified peaks appear after a long exposure to 2% Chlorhexidine, the polymer additives in the formulation may be eluted, the product may have some chlorhexidine left over, or there may have been an interaction product present. Evidence previously demonstrated that chlorhexidine can cause material-dependent chemical changes such as surface deterioration, corrosion and ion release from dental materials. In addition, chlorhexidine can be stored inside or integrated into polymeric structures, providing the possibility for continued interaction after the initial exposure. The peaks detected, however, were not identified as particular compounds without confirmatory analysis by LC-MS or GC-MS (19, 20).

Both materials became rougher over time, especially following the 2% chlorhexidine for long-term use. In the early stages of the study, polyamide was rougher than PMMA, and this difference persisted during the study, in line with the well-known challenges of finishing and polishing flexible denture polymers. Chronic

exposures can leach out superficial matter, dissolve soluble materials or cause micro perturbations. Changes as a result of long exposure to denture cleansers and disinfecting solutions have been reported similar to those described here (7, 9, 12, 15, 16).

This is because the roughness observed after long-term exposure to chlorhexidine could be clinically relevant due to the potential to hold stains on its rough surface and the protection that may afford to receptive sites for the attachment of microorganisms. Other factors, however, such as surface energy, hydrophobicity, formation of salivary pellicle, microbial species and hygiene practices can also influence microbial colonisation. The present study did not directly measure biofilm formation, but the increase in roughness may indicate a greater likelihood of more microbes being retained in the area; that does not necessarily mean more clinical biofilm formation (7, 8, 9, 15).

Microhardness and flexural strength were found to be inversely proportional to chlorhexidine concentration and chlorhexidine exposure time. The molecules of water and disinfectant can enter the polymer matrix, leading to an increase in the distance between chains, causing a plasticising effect and decreasing the resistance to indentation and bending. This is supported by the concurrent rise in water sorption, solubility and release of leachate. Other studies have also found evidence of mechanical property loss after extended exposure to chemical cleansers, but the amount of loss will vary by polymer composition and immersion procedure (5, 6, 11, 15, 16, 17, 18).

Polyamide had lower flexural strength than PMMA but showed greater deformation before failure, reflecting its more flexible behaviour. The differences are due to the firmness of PMMA and the flexibility of polyamide. The PMMA values after lengthy exposure to 0.12 % chlorhexidine were still within a generally acceptable range, while 2 % chlorhexidine caused a more pronounced decrease, indicating that the regular use of higher concentration may compromise the mechanical safety margin of an aged and/or poorly supported denture. However, clinical failure also relies on the thickness of the prostheses, their fit, occlusal loading, porosity, and accidental impact, as well as the repair history of the prostheses (1, 5, 6, 11).

The results of cytocompatibility revealed that after 6 months of repeated exposure to chlorhexidine, there was a decrease in the viability of gingival fibroblasts. The extraction of PMMA had a more significant effect than the extraction of polyamide, with a higher effect value after 2% chlorhexidine

was applied for a longer period. The mean viability of only the PMMA group treated with 2% chlorhexidine for the simulated period fell below the 70% cytotoxicity limit. This effect could have been due to any of the residual methyl methacrylate, remaining chlorhexidine, unknown extractable compounds or a mix of several. Viability is strongly correlated with the amount of methyl methacrylate released, which indicates a relationship but does not prove that methyl methacrylate is the only component responsible for the cell response (3, 4, 13, 14, 21).

Polyamide has more water sorption and surface roughness, but has better cell viability. The difference in the cytocompatibility of the two materials observed indicates that polyamide could be a better choice for patients with known sensitivity to acrylic monomer; however, clinical confirmation is needed. However, polyamide should not be viewed as biologically inert since its response is dependent on its formulation, processing and ageing conditions. More fluid absorption can also lead to the accumulation and release of a substance from the surroundings of the material (2, 22, 23).

The 70% viability is a useful figure for screening *in vitro* but does not necessarily translate to clinical tissue injury. In the oral cavity, compounds released from tooth surfaces are diluted by saliva, which is constantly replaced, and mucosa has repair mechanisms which are lacking in a cell culture model. However, exposure may be higher in some patients if they wear a denture for a long period of time and have reduced saliva production. So, the observed reduction in viability should be viewed as a warning signal, which should be confirmed in a clinical setting, not as proof of clinical toxicity (14, 21).

The relationships between the leachate release, water sorption, roughness, mechanical deterioration and cell viability indicate that they are related aspects of polymer ageing. Immersion in the medium can cause structural changes that can lead to surface and mechanical degradation and can increase the mobility of the chains. Structural changes arising during the immersion can cause mechanical and surface damage as well as increasing the mobility of the chains. The correlations do not imply a causal sequence; however.

The results from a clinical point of view do not suggest that chlorhexidine should be avoided altogether. It is still an effective antimicrobial agent to control microorganisms associated with dentures. Routine denture care with chlorhexidine, however, could be inappropriate with prolonged

use for a day's denture. Lower concentrations or lower concentrations for shorter durations or intermittently followed by a thorough rinse may offer a more satisfactory combination of microbial control and preservation of material integrity. The type of denture base should also be taken into consideration, since there was no universal response from PMMA and polyamide (7, 8, 9, 10, 13).

A major limitation is that the retention of residual chlorhexidine in the PMMA and polyamide specimens cannot be quantified. There is potential that the cytotoxic effects observed were due to separate action of retained chlorhexidine, apart from polymer derived leachates. Future studies should quantify residual chlorhexidine, in order to differentiate disinfectant-derived effects from material-derived biological effects. The major strengths of the present study were the simultaneous evaluation of the chemical, surface, mechanical, and biological properties, the use of two denture base materials, and the use of two concentrations of chlorhexidine in a standardised six-month exposure protocol. The *in vitro* design, the employment of only one commercial product for each material category, and the absence of oral temperature changes, mastication, brushing, diet, pH changes, and microbial activity are limitations. Chromatographic peaks were not identified by mass spectrometry, and the amount of chlorhexidine that remained was not determined separately. Other commercial materials, additional thermal cycling, mechanical brushing, cyclic loading, chlorhexidine analysis, and direct testing of biofilms should be included in future studies.

Conclusion

Repeated chlorhexidine disinfection for six months resulted in concentration-dependent changes in both polymethyl methacrylate (PMMA) and polyamide denture base materials. Exposure to chlorhexidine affected an increment in surface roughness, water sorption, solubility and material-related leachate release, and a decrease in microhardness, flexural strength, and gingival fibroblast viability. Although PMMA had the smoothest surface and the highest flexural strength, it emitted more amounts of MMA, and it induced more decrease in the viability of the fibroblasts. The polyamide material was more cytocompatible, while it had higher water sorption and surface roughness. Although moderate changes occurred with 0.12% chlorhexidine, 2% chlorhexidine resulted in more pronounced chemical, mechanical and biological degradation after 6 months. Routine daily immersion in 2%

chlorhexidine should be avoided, in favour of using lower concentration and/or less frequent immersion, or of alternative immersion methods, especially for PMMA-based dentures.

Study limitations

This study was in vitro, did not utilise thermal cycling, mechanical loading, brush and food pH changes, nor saliva flow or the formation of biofilm, and did not measure the quantity of residual chlorhexidine found in the specimens. So clinical generalisation cannot take place directly without clinical validation.

Declarations

Ethics approval and consent to participate

The study was done in vitro, with human gingival fibroblast cells obtained from a commercial source. There were no human subjects and no identifiable clinical samples used directly, and so formal ethical approval was not necessary as per institutional guidelines.

Consent for publication

This paper is published under the Creative Commons Attribution-Noncommercial 4.0 International License with the cooperation of all authors.

Data availability

The datasets used and/or analysed in the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MSA contributed to the study concept, design, and supervision. KIF performed specimen preparation and laboratory testing. AFNMT contributed to cell-culture procedures and data collection and performed the statistical analysis. All authors contributed to interpretation of the findings, drafting and revising the manuscript, and approved the final version.

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Artificial intelligence disclosure

Generative artificial-intelligence tools were used only for language editing and improvement of readability. The authors reviewed and verified all scientific content, experimental data, statistical analyses, interpretations, and final revisions, and are ultimately responsible for the manuscript.

Abbreviations

CHX: Chlorhexidine.

FTIR: Fourier Transform Infrared Spectroscopy

HPLC: High-Performance Liquid Chromatography

LC-MS: Liquid Chromatography-Mass Spectrometry.

GC-MS: Gas chromatography-mass spectrometry

MMA: Methyl methacrylate

PMMA: Polymethyl methacrylate

Ra: Arithmetic mean surface roughness

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