

Effect of Ionic Liquids on Mechanical, Physical, and Antifungal Properties and Biocompatibility of a Soft Denture Lining Material

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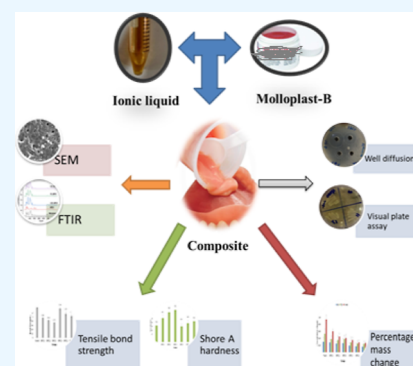
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ABSTRACT: This study aims to evaluate the effect of ionic liquids and their structure on the mechanical (tensile bond strength (TBS) and Shore A hardness), mass change, and antifungal properties of soft denture lining material. Butyl pyridinium chloride (BPCL) and octyl pyridinium chloride (OPCL) were synthesized, characterized, and mixed in concentrations ranging from 0.65–10% w/w with a soft denture liner (Molloplast-B) and were divided into seven groups (C, BPCL1-3, and OPCL1-3). The TBS of bar-shaped specimens was calculated on a Universal Testing Machine. For Shore A hardness, disc-shaped specimens were analyzed using a durometer. The mass change (%) of specimens was calculated by the weight loss method. The antifungal potential of ionic liquids and test specimens was measured using agar well and disc diffusion methods ($p \leq 0.05$). The alamarBlue assay was performed to assess the biocompatibility of the samples. The mean TBS values of Molloplast-B samples were significantly lower ($p \leq 0.05$) for all groups except for OPCL1. Compared with the control, the mean shore A hardness values were significantly higher ($p \leq 0.05$) for samples in groups BPCL 2 and 3. After 6 weeks, the OPCL samples showed a significantly lower ($p \leq 0.05$) mass change as compared to the control. Agar well diffusion methods demonstrated a maximum zone of inhibition for 2.5% OPCL (20.5 ± 0.05 mm) after 24 h. Disc diffusion methods showed no zones of inhibition. The biocompatibility of the ionic liquid-modified sample was comparable to that of the control. The addition of ionic liquids in Molloplast-B improved the liner's surface texture, increased its hardness, and decreased its % mass change and tensile strength. Ionic liquids exhibited potent antifungal activity.



1. INTRODUCTION

Denture liners are relining materials that are applied to the fitting surface of a denture to improve the fit of the denture or decrease the masticatory forces transmitted to the oral mucosa.¹ It is broadly classified into three types: hard reliners, soft liners, and tissue conditioners.² Hard reliners are not used nowadays because they lose their elasticity within short periods and cause irritation to the oral mucosa.³ Tissue conditioners have limited use in prosthodontics as they require changing every 3–5 days.⁴

The term soft liner refers to the resilient denture base lining material that is used to reline the intaglio surfaces of a removable denture. The main indications for soft liners include knife edge alveolar ridges, atrophied mucosa, and patients who cannot tolerate stresses induced by dentures.⁵ They provide a cushioning effect. Based on durability, soft liners are broadly categorized into short-term and long-term, and based on the chemical composition the long-term soft liners are grouped into acrylic resin and silicone elastomers. Silicone elastomers, which typically consist of polymers of dimethyl siloxane, do not rely on leachable plasticizers and hence remain elastic for a long time.⁶

Since the introduction of silicone soft denture liners, they have been widely used as they provide a more economical and less invasive treatment alternative for improving patient comfort and fit of the denture.⁷ Despite their extensive use, none of the available materials could meet the ideal requirements, which include adequate bonding, less fluid uptake, inhibition of microbial growth, and tear resistance.⁶ Molloplast-B, which is a commercially available silicone-based soft denture lining material, possesses major limitations in terms of debonding from the denture base, fungal colonization, and fluid uptake.⁸ This study aims to address these issues by adding ionic liquids to Molloplast-B.

Ionic liquids are organic salts with anions and cations and melting temperatures of less than 100 °C.⁹ Pyridinium-based molten ionic liquids contain heterocyclic structures with

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different functional groups attached either to the pyridine ring or nitrogen atom. Primarily, they were used as an antimicrobial agent, but later researchers modified the structure of pyridinium-based ionic liquids and made them versatile and applicable to a wide variety of applications like catalysis,¹⁰ solvent and surfactant,^{11,12} corrosion inhibition,¹² cellulose processing,¹³ functionalized polymeric membrane,¹⁴ coordination polymer,¹⁵ drug delivery,¹⁶ cosmetics,¹² etc. Therefore, it is hypothesized that the addition of an ionic liquid (alkylpyridinium chloride-based, alkyl; octyl group—hydrophobic and antifungal, butyl group—hydrophobic and less antifungal) and the chloride anion (known antimicrobial and reactive species) will improve the adhesion of the liner (silicon-based) with the denture (PMMA), decrease the uptake of fluids by the material, and make it less prone to fungal colonization. The ionic liquids were synthesized and mixed in different ratios with Molloplast-B lining materials. The composite was analyzed for the physical texture with scanning electron microscopy (SEM) and chemistry with Fourier transform infrared (FTIR) spectroscopy. The composite was also evaluated for Shore A hardness and tensile bond strength. Water uptake analysis for the composite was also performed, and the composite's antifungal properties were assessed through disc diffusion, well diffusion, MIC, and visual streaking methods against *Candida albicans*.

2. METHODOLOGY

2.1. Materials. The materials used in this study are tabulated in Table 1. Molloplast-B (10,216) was bought from

Table 1. List of Materials Used in This Study

material	company	CAS/CAT number
1. Molloplast-B	Detax	10,216
2. pyridine	Sigma-Aldrich	110-86-1
3. 1-butyl chloride	Sigma-Aldrich	109-69-3
4. octyl chloride (1-chloro octane)	Sigma-Aldrich	111-85-3
5. Sabouraud's dextrose agar (4%)	Merck	105,438
6. Mueller Hinton agar (nutria select)	Merck	103,872
7. <i>Candida albicans</i> strain	ATCC	10,239
8. polymethyl methacrylate	Sigma-Aldrich	9011-14-7
9. methyl methacrylate	Sigma-Aldrich	80-62-6
10. RPMI culture media	Sigma-Aldrich	R8758

Detax, and the *Candida* fungus strain (ATCC 10239) was obtained from the American Type Culture Collection and stored in a refrigerator at 4 °C as recommended. Most of the materials were bought from Sigma-Aldrich and Merck.

2.2. Synthesis of Ionic Liquids. **2.2.1. Synthesis of Butyl Pyridinium Chloride.** Butyl pyridinium chloride (BPCl) was prepared by the method described in our earlier publication.¹³ 40.2 mL of pyridine (110-86-1 Sigma-Aldrich) was mixed with 79.5 mL of 1-chlorobutane in a 250 mL three-neck round-bottom flask. The mixture was heated for 48 h with continuous stirring until a viscous product was obtained. The final product was washed with ethyl acetate, which removed the unreacted products of pyridine and chlorobutane, as they are soluble in ethyl acetate. FTIR (ν_{\max} , cm^{-1}): 3057, 2963, 2875, 1634, 1489, 1171. ¹H NMR (D_2O): δ 0.88 (triplet, $J = 7.2$, 3H), 1.30 (sextet, $J = 7.2$ –7.6, 2H), 1.94 (quintet, $J = 7.2$ –7.6, 2H), 4.56 (triplet, $J = 7.2$, 2H), 8.03 (triplet, $J = 6$, 2H), 8.49 (triplet, $J =$

7.6, 1H), 8.81 (doublet, $J = 6$, 2H), FTIR and NMR spectra are shown in Figures 1 and 2, respectively.

2.2.2. Synthesis of Octyl Pyridinium Chloride. For the preparation of octyl pyridinium chloride (OPCL) molten salt, 5 gram of Pyridine and 10.3 gram of 1-chlorooctane were mixed in a 100 mL flask. This mixture was kept for stirring for 1 h at room temperature. This mixture was then heated to 110 °C and stirred continuously for 30 h. The final product obtained was washed with ethyl acetate to remove the unreacted pyridine. FTIR (ν_{\max} , cm^{-1}): 3042, 2924, 2855, 1714, 1634, 1488, 1159, 777. ¹H NMR (D_2O): 0.75 (3H, app. triplet, $J = 8$ Hz), 1.10–1.30 (10H, m), 1.88–1.98 (2H, m), 4.52 (3H, triplet, $J = 7.2$ Hz), 7.98 (2H, app. triplet, $J = 6.8$ Hz), 8.45 (1H, triplet, $J = 7.6$ Hz) 8.74 (2H, doublet, $J = 5.6$ Hz),¹³ FTIR and NMR spectra are shown in Figures 3 and 4 respectively.

2.3. Preparation of Composites. The ionic liquids BPCl and OPCL were added in different concentrations w/w % to Molloplast-B; details are given in Table 2. The required amounts of Molloplast-B and ionic liquids were weighed in a digital balance (Shimadzu Electronic Balance, Japan) and mixed on a non-absorbent mixing pad using a spatula for 2–3 min.

2.4. Characterizations. **2.4.1. Scanning Electron Microscopy.** The surface structure of the disc samples prepared was analyzed using a scanning electron microscope (JSM-IT-100, Jeol, Japan). The samples were first coated with gold in a gold sputter (QUORUM) for 90 s at a 10 mA current. The SEM analysis was done to examine the surface homogeneity and porosities. The images were taken at magnifications of 100 \times , 1000 \times , and 3000 \times , with the voltage kept at 20 kV.

2.4.2. FTIR Spectroscopy. The FTIR spectroscopy of the control and experimental group samples, along with the obtained ionic liquid, was done using ATR as an accessory coupled to the FTIR spectrometer (Nicolet IS 50, Thermo Fischer Scientific USA). The spectra were collected over the region of 4000–400 cm^{-1} . The data was interpreted using OMNIC software.

2.5. Mechanical Testing. For mechanical tests, five samples each for the experimental and control groups were evaluated.

2.5.1. Shore A Hardness. Disc samples with dimensions of 6 mm diameter and 2 mm height were used to evaluate the Shore A hardness. The hardness of all the specimens was assessed using a WESTOP Type-A durometer (Nishi Tokyo Seimitsu Co.Ltd. Japan) according to specifications described in ISO 7619-1¹⁷ (12). The samples were stored in distilled water at 37 °C for 24 h before measuring the hardness. After 24 h, the samples were placed on a flat and hard surface, and the foot of the indenter was slowly lowered into the sample until the sample was touched.¹⁸ The hardness of the cured samples was measured after loading for 5 s. The measurements were recorded at three different points.

2.5.2. Tensile Bond Strength. The bar samples with dimensions of 83 mm length, 10 mm height, and 10 mm width were used to evaluate the tensile bond strength (TBS) according to ISO specifications ISO 10139-2.¹⁸ The samples were placed in a vertical alignment in a UTM (Autograph AG-IS SHIMADZU, Japan). The samples were tested under tensile force at a crosshead speed of 5 mm/min under a load cell of 5 kN until bond failure occurred. The maximum force before bond failure was recorded by UTM. The cross-sectional area of the sample was assessed using a Vernier caliper.

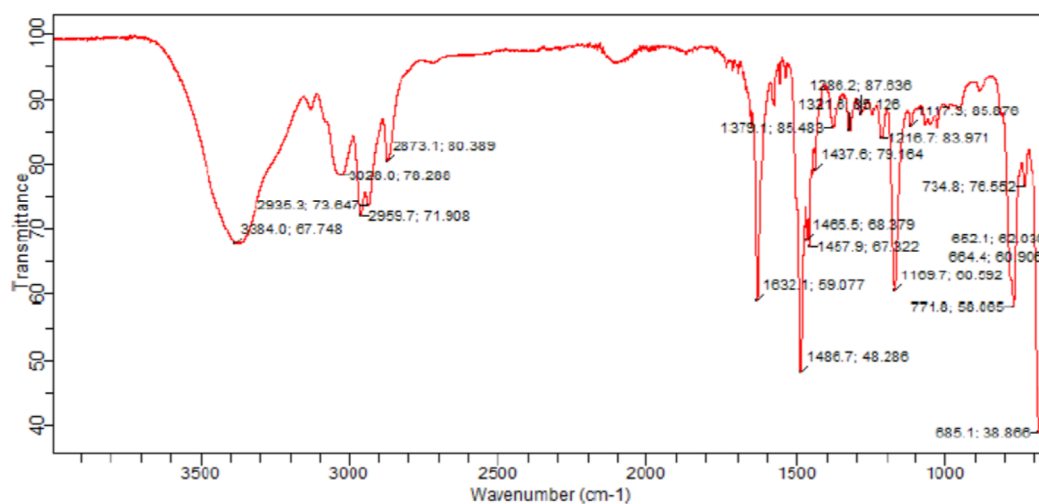


Figure 1. FTIR spectrum of BPCL.

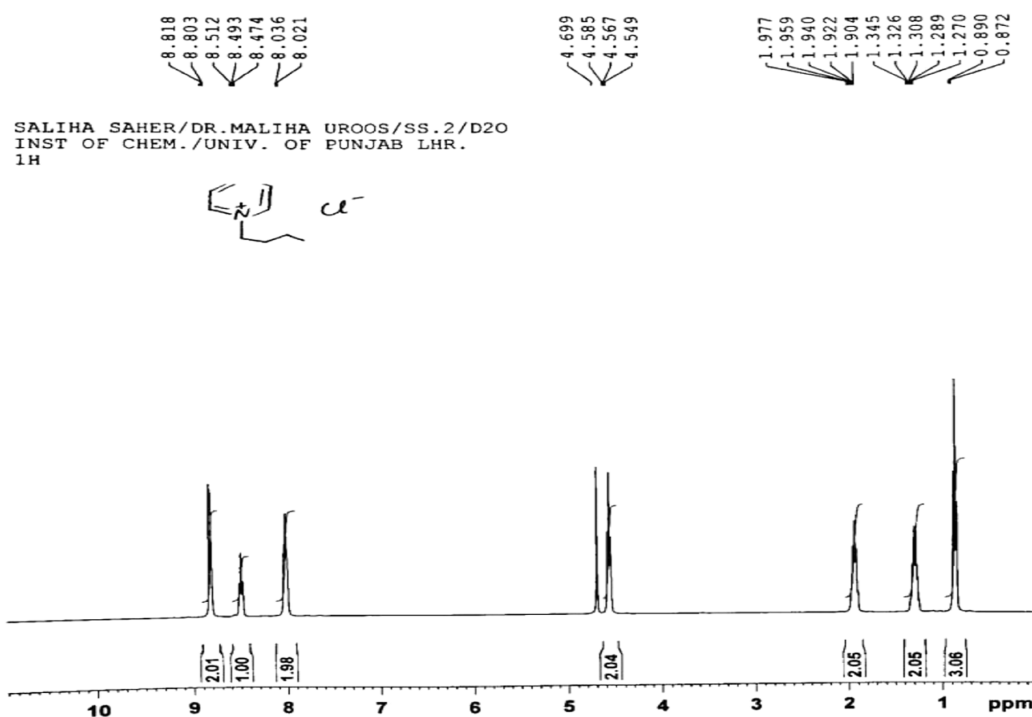


Figure 2. ^1H NMR spectrum of BPCL.

The bond strength of the sample was calculated using the following formula

$$\text{bond strength} = F/A \quad (1)$$

where F is a force before bond failure and A is the cross-sectional area.

2.6. Physical Testing. For physical tests, three samples were evaluated for each of the experimental and control groups.

2.6.1. Percent Mass Change. For percent mass change, disc samples with dimensions of 6 mm diameter and 2 mm height were used, and the test was performed according to ISO specification 10139-2.¹⁸ The samples from each group were weighed on a digital balance. Eppendorf tubes were taken and labeled with the concentrations of different experimental groups and control groups. The tubes were then filled with

distilled water, and the weighed samples were then immersed in distilled water within the Eppendorf tubes. The samples were weighed at 24, 48, and 72 h and then weighed weekly. The % mass changes were measured for 6 weeks.¹⁹

The percent mass change was calculated according to the following formula

$$\text{percentage mass change} = \frac{(W_2 - W_1) \times 100}{W_1} \quad (2)$$

where W_1 = initial weight (before water immersion), W_2 = weight after water absorption, and W_3 = final weight.

2.7. Antifungal Testing. For antifungal tests, three-disc samples for all the groups were evaluated.

2.7.1. Disc Diffusion and Agar Well Diffusion Method. A solution was prepared by mixing 3.8 g of Mueller Hinton Agar (MHA) powder in 100 mL of water while maintaining a pH of

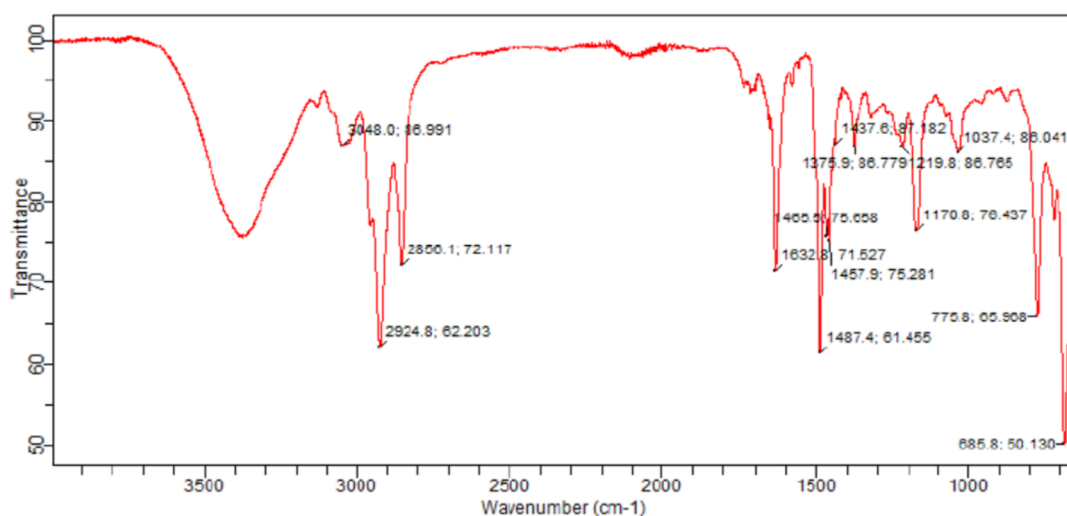


Figure 3. FTIR spectrum of OPCL.

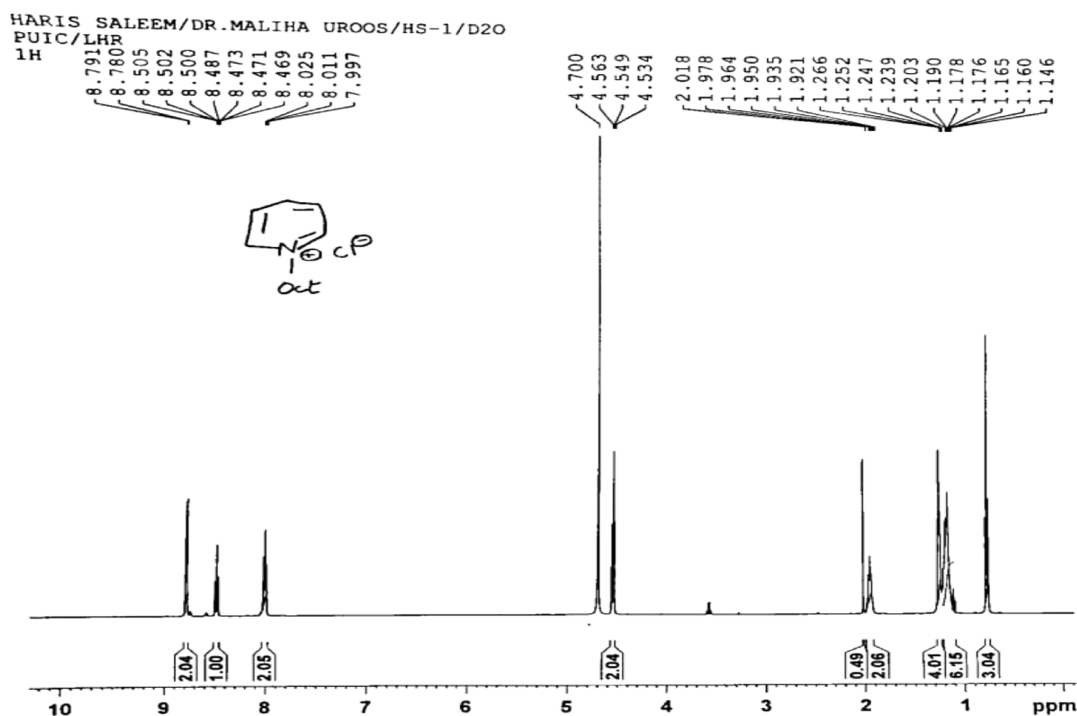


Figure 4. ^1H NMR spectrum of OPCL.

Table 2. Group's Distribution along with the Concentrations of Molten Salts

groups	composition
control	Molloplast (commercially available silicone soft denture liner)
BPCL 1	2.5% BPCL+ 97.5 %Molloplast-B
BPCL 2	5% BPCL+ 95%Molloplast-B
BPCL 3	10% BPCL+ 90%Molloplast-B
OPCL 1	0.65% OPCL+ 99.35%Molloplast-B
OPCL 2	1.25% OPCL+ 98.75%Molloplast-B
OPCL 3	2.5% OPCL+ 97.5%Molloplast-B

7.4. The solution was autoclaved at 121 °C for 15 min. After cooling, 20 mL of the solution was added to Petri dishes in a sterile environment. The surface of the Petri dishes was then inoculated with *Candida albicans* (ATCC 10239) suspension

of 10^7 UFC/mL using a sterile swab. For the disc diffusion tests, the discs prepared from composite material were placed on the inoculated media contained in the Petri dishes along with a control. The dishes were incubated at 37 °C for 24 h to provide suitable growth conditions for the bacteria. The zone of inhibition was measured in mm using a Vernier caliper or ruler and compared with the control for antifungal potency.

For the well diffusion method, the already prepared and inoculated Petri dishes were used. Wells were made with an 8 mm well borer. Ionic liquids were dissolved in distilled water to make the concentrations used for the experimental groups discussed in Table 1. 100 microliters of each concentration were added to the wells, followed by the incubation of Petri dishes at 37 °C for 24 h.²⁰

2.7.2. Minimum Inhibitory Concentration. To determine the MIC, the double dilution method was used. The procedure

followed was according to the guidelines given by CLSI.²¹ The highest effective concentration of the ionic liquids used in the well diffusion method was taken, i.e., 2.5% for octyl pyridinium chloride and 10% for BPCL.

For making solutions, 0.025 g of OPCL salt was taken and dissolved in 1 mL of pure DMSO (dimethyl sulfoxide), and 0.1 g of BPCL was taken and dissolved in 1 mL of pure DMSO.

These solutions were then further diluted by taking 500 μL from the first tube along with 500 μL of DMSO. Both salts were diluted up to thirteen times through this method. 96-well plates were taken, and in the first well of the first row 200 μL of only broth, the medium was added (positive control), and in the twelfth well of the first row, 200 μL of 0.5 McFarland suspensions was added. In the wells, 2nd–11th of the first row 100 μL of BPCL was added in different concentrations ranging from higher to lower. 100 μL of 0.5 McFarland suspensions were also added to the wells 2nd–11th, as shown in Figure 5.

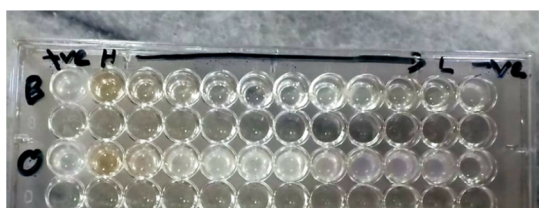


Figure 5. 96-well plates with various concentrations of ionic liquids.

The same procedure was repeated for 2nd row, but instead of BPCL, OPCL was used. The 96-well plates were then incubated at 37 °C for 24 h. After 24 h, the turbidity was checked, but since the compound was colored, a visual plate assay was also performed for confirmatory purposes. For the visual plate assay, MHA media was prepared first, and then, 1 drop from each well was taken and streaked on the prepared media along with negative and positive controls. The MHA plates were then incubated for 24 h at 37 °C and after 24 h the growth on different plates was assessed.

2.8. Biocompatibility Assay. The cytocompatibility and cell viability of the control (Molloplast-B and samples (Molloplast-B with 0.65% octyl pyridinium chloride, 1.25% octyl pyridinium chloride, 2.5% butyl pyridinium chloride, 5% butyl pyridinium chloride, and 10% butyl pyridinium chloride) were determined by performing the cytotoxicity assay with the Alamar blue reagent using NIH 3T3 fibroblasts. The samples were first sterilized with 70% ethanol and then washed with PBS thrice before the experiment). Then the assay was performed by direct incubation of cells with control and composite scaffolds in different concentrations (0.65, 1.25, 2.5, 5, and 10%) in a 24-well cell culture plate for 24 h. After 24 h, the medium was replaced by Alamar blue reagent and incubated for 4 h at 37 °C. After 4 h, the absorbance was measured by a microplate reader at 570 nm.

2.9. Statistical Analysis. All collected data were entered into a Microsoft Excel spreadsheet in 2016 and transferred to SPSS 22 for analysis. Basic data analysis like the mean and standard deviation was calculated for numerical variables like Shore A hardness, TBS, and antifungal zones of inhibition. These variables were compared among control and experimental groups containing various concentrations of ionic liquids (0.65, 1.25, and 2.5% for OPCL and 2.5, 5, and 10% for BPCL) in soft denture liners using one-way ANOVA in SPSS 22. In cases of significance post hoc analysis was done by using

the Tukey test in SPSS 22. For all analyses, the level of significance was $p \leq 0.05$. For visualization of data, bar graphs were made in Microsoft Excel spreadsheet 2016.

3. RESULTS AND DISCUSSION

In this study, we used a commercially available permanent silicone-based soft denture liner, Molloplast-B, that comprises polydimethylsiloxane with benzoyl peroxide as an initiator. It is an odorless, tasteless, and single component soft liner that is available in the preformed dough. Due to their major drawbacks of debonding²² from the denture base and fungal growth,⁸ two different ionic liquids, i.e., BPCL and OPCL ionic liquids were added in different concentrations ranging from 0.65–10% in Molloplast-B to improve the inferior properties of soft denture liners. The characterization of freshly prepared composites and the control group was done along with evaluation of mechanical, physical, and antifungal properties.

3.1. Characterizations. **3.1.1. Scanning Electron Microscopy.** A variety of microscopic techniques exist for the structural characterization of polymers and composites, but SEM is one of the fundamental tools used in the fields of biomedicine, dental science, and research for the structural characterization of polymers. These images provide details about the surface structure and composition of the material, along with the crystalline structure and dispersion of particles.²³ In this study, the SEM images were taken at magnifications of 1000 \times and 3000 \times to analyze the difference between the surface morphology of the control and experimental groups. The SEM image of the control group, as shown in Figure 6a, correlates with a previous study.²⁴ Figure 6b–g shows the conglomerate structure of the experimental groups caused by the addition of ionic liquids in Molloplast-B. Compared with the control group, all the experimental groups showed a decrease in porosity and an increase in surface homogeneity. An increase in flake-shaped structures was observed as the concentration of octyl pyridinium increased. The rod-shaped structure of glass fibers is seen in all micrographs.

3.1.2. FTIR Spectroscopy. FTIR spectroscopy is a universally accepted tool for analyzing the chemical structure of a material at a molecular scale.²⁵ Figures 7 and 8 show various FTIR spectra obtained for control and experimental groups. For cured Molloplast-B, the characteristic peaks were obtained at (C–H) in CH_3 at 2942 cm^{-1} , (C–H) in Si– CH_3 at 1260 cm^{-1} , (Si–O–Si) in Si–O–Si at 1091 cm^{-1} , (Si–O–Si) in Si–O–Si at 1023 cm^{-1} , and (C–H) in Si– CH_3 at 801 cm^{-1} . In the case of ionic liquids, for BPCL, the peaks were recorded at (C–H) 3057 cm^{-1} , 2963 cm^{-1} (C–H), 2875 cm^{-1} , (C=C stretching) 1634 cm^{-1} , (C–H) 1489 cm^{-1} , and (C–O) 1171 cm^{-1} , while for OPCL ionic liquid, the peaks were recorded at (O–H) 3042 cm^{-1} , (C–H) 2924 cm^{-1} , (C–H) 2855 cm^{-1} , (C–H) 1714 cm^{-1} , (C=C stretching) 1634 cm^{-1} , (C–H bending) 1488 cm^{-1} , (C–O stretching) 1159 cm^{-1} , and (C–H bending) 777 cm^{-1} . The FTIR spectra recorded for the Molloplast, OPCL, and BPCL were similar to peaks recorded in previous studies.^{13,26} Characteristic peaks of Molloplast-B at 1092 and 1023 cm^{-1} represent the siloxane bonds (Si–O–Si and Si– CH_3). For ionic liquids, a weak C–H bend observed at 1632 cm^{-1} for BPCL and at 1633 cm^{-1} is indicative of an aromatic compound, confirming the presence of a pyridine ring in the molten salts. In the experimental groups, as shown in Figures 7 and 8, the peaks of the ionic liquids are not visible in the recorded FTIR spectra. This is

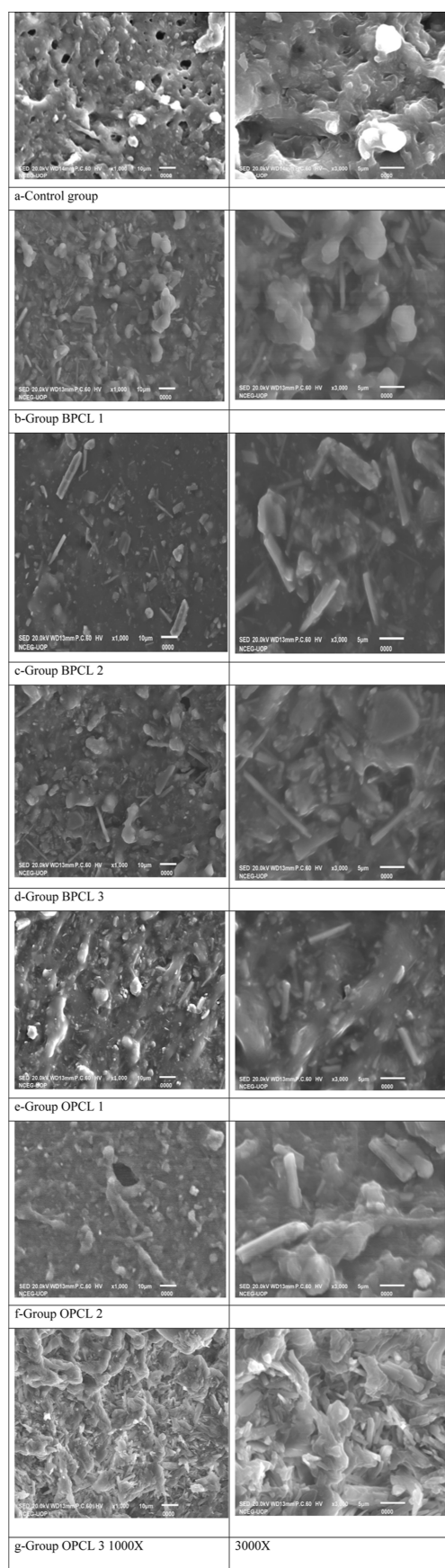


Figure 6. (a–g) Shows SEM images of control and experimental groups at 1000X and 3000X.

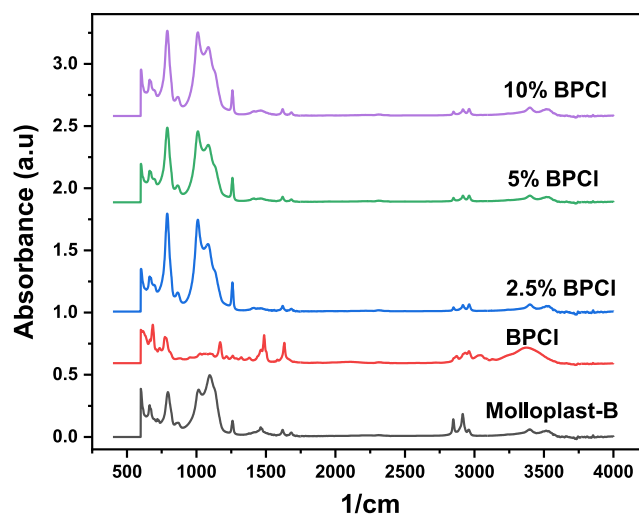


Figure 7. FTIR spectra of Molloplast-B compared with butyl pyridinium-containing groups.

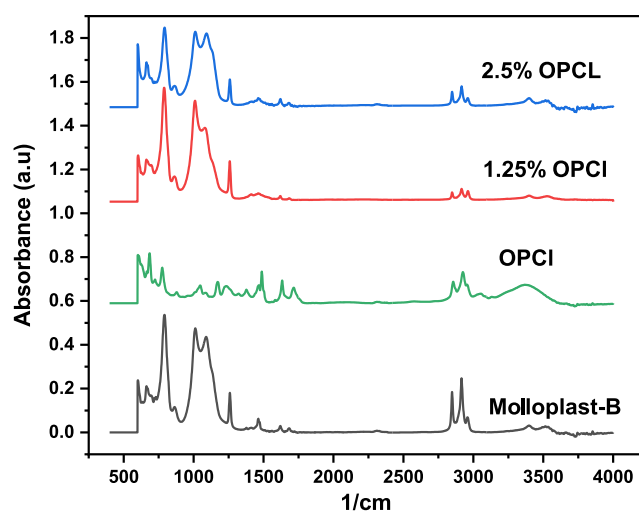


Figure 8. FTIR spectra of Molloplast-B compared with octyl pyridinium-containing groups.

because the peaks are overlapped by the peaks of Molloplast-B, and the concentration of the ionic liquid is also very low.

3.2. Mechanical Testing. The durability and success of the material are mainly dependent on its mechanical properties; therefore, in our study, we evaluated the shore A hardness and bond strength of modified and unmodified Molloplast-B.

3.2.1. Shore A Hardness. The shore A hardness test result showed that the maximum hardness was observed for the BPCL 3 group (47.2 ± 0.74 shore units), while the minimum values were observed for the OPCL 1 (43.0 ± 0.74 shore units), as shown in Figure 9. Compared with the control group, only OPCL 1 showed a decrease in hardness, while all other experimental groups showed an increase in hardness with increasing concentrations of the molten salts. A one-way ANOVA showed that there was a significant difference ($p \leq 0.05$) between the groups. Hardness is the ability of a material to resist indentation. The shore A hardness is used to evaluate the hardness of rubber-like materials.²⁷ Hardness is directly related to the elastic property of a material so that it can evenly distribute forces and increase patient comfort.²⁸ For soft

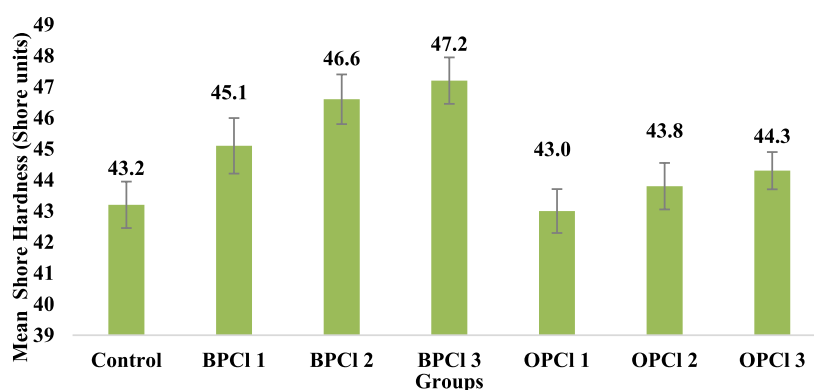


Figure 9. Shore A hardness values with error bars (SD) for control and experimental groups.

denture liners, lower values of hardness are desirable, and therefore, ISO 10139 part-2 classifies these materials as either extra soft (Shore A hardness ≤ 25) or soft (Shore A ≤ 50).¹⁸ So, following these criteria, the control and all the experimental groups fall into the category of soft denture liners and can be recommended for use in clinical practice. The initial hardness of Molloplast-B recorded was almost similar to the results obtained by Canay et al.²⁹ The experimental groups, however, showed a gradual increase in hardness with an increasing percentage of ionic liquids, as shown in Figure 4, except that the OPCL 1 group showed similar results to the control group. The increase in hardness in experimental groups may be due to the increase in crosslinking of polymers, resulting in increased density and hardness, as concluded from a study by Maciejewska and Sowinska.³⁰ They studied the effect of fillers and ionic liquids on the crosslinking of natural rubber, and they also observed an increase in the shore hardness of the rubber compared to the control when butyl imidazolium bromide and butyl pyrrolidinium were incorporated into the rubber. Another study also concluded that the addition of ionic liquids to rubber increased its crosslink density, which led to an increase in hardness.³¹

3.2.2. Tensile Bond Strength. The results of TBS show that the addition of ionic liquids negatively impacted the TBS. The control group showed the maximum (1.46 ± 0.06 MPa) TBS, while the lowest (0.73 ± 0.02 MPa) TBS was recorded for the BPCL 3 group that contained the highest percentage of BPCL. Compared with the control group, all experimental groups showed a decrease in TBS, as shown in Figure 10. One-way ANOVA showed that there was a statistical significance (p -value ≤ 0.05) among all groups. The bonding between the soft denture liner and denture base is crucial for its long-term

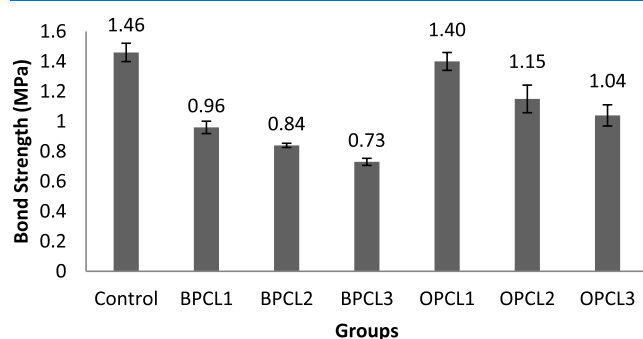


Figure 10. TBS with error bars (SD) for control and experimental groups.

functioning and stability. Several methods exist to evaluate the bond strength, such as the peel test and shear bond strength, but the most widely accepted and used is the tensile test method, as recommended by ASTM and ISO,²⁸ which is also used in this study. According to previous studies,^{32,33} a bond strength of 0.44 MPa is acceptable for clinical use. According to this criterion, all the samples from control and experimental groups can be considered for use in clinical practice. However, the addition of BPCL and OPCL to Molloplast-B decreased the bond strength. The bond strength decreased as the concentration of ionic liquids increased. The pyridine-based ionic liquids are also used as cationic surfactants (pyridine ring: head and alkyl chain attached to nitrogen of pyridine: tail); hence, this decrease in bond strength could be attributed to the surfactant property of pyridinium-based ionic liquid, which has altered the interface of the lining material (decrease the adhesive force and increase cohesive force) concerning the material of the denture surface in TBS measurement. This decrease in bond strength is also validated by the study done by Anna Marzec, which attributed the decreased TBS to a poor interface between the soft denture liner and denture base.³¹ This decrease can also be attributed to the polymerization method used, as studies suggest higher bond strength when PMMA and soft denture liner are processed together.³⁴ The bond strength is also influenced by other factors such as the thickness of the soft liner, the cross-head speed of the UTM, the material used for denture base fabrication, and the test method used;¹⁸ therefore, a single test method cannot be used for an accurate analysis of bond strength.

3.3. Physical Testing. The physical properties of a material are equally important for its proper functioning. Water uptake, which is an inherent property of porous materials, is also an issue that needs to be addressed with soft denture liners, so we evaluated the effect ionic liquid had on the percentage mass change of Molloplast.

3.3.1. Percentage Mass Change. The results showed that the least percent mass change was observed in groups containing OPCL, followed by BPCL, and the control group showed the highest percentage of mass change. The graph in Figure 11 shows that the highest % of mass change was observed for the control group, and the least was found for the OPCL3 group after 24, 72 h, and 1 week.

The rate of diffusion with which all the samples continued to increase mass from day 1 to 6 weeks of immersing samples in distilled water is shown in Figure 12. On day 1, the highest value of the rate of mass change was recorded for the control group, and the lowest rate was expressed by the group OPCL

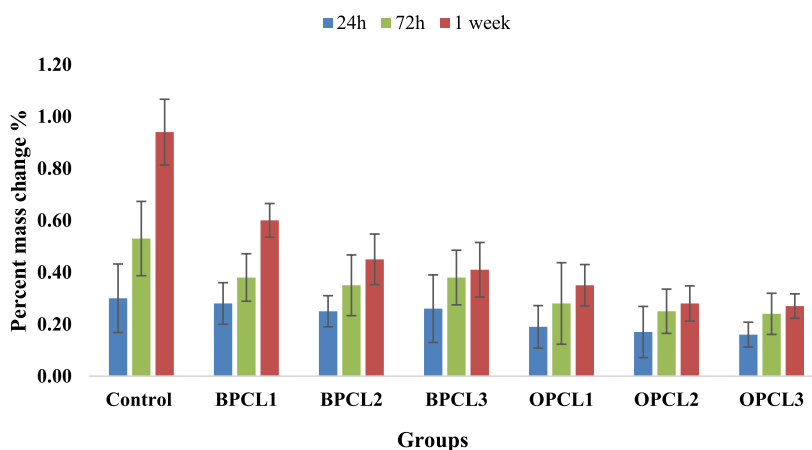


Figure 11. Percentage mass change with error bars (SD) for control and experimental groups after 24, 72 h, and 1 week.

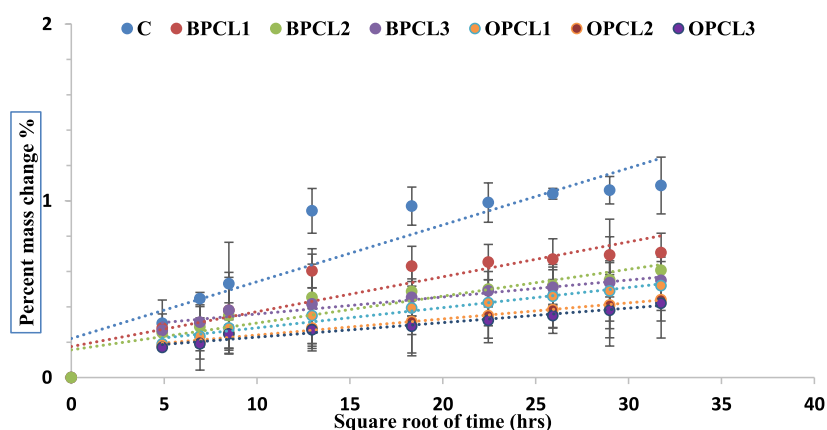


Figure 12. Rate of % mass change for 24, 48, 72 h, and week 1 to week 6 for the control and all the experimental groups plotted against the square root of time in hours.

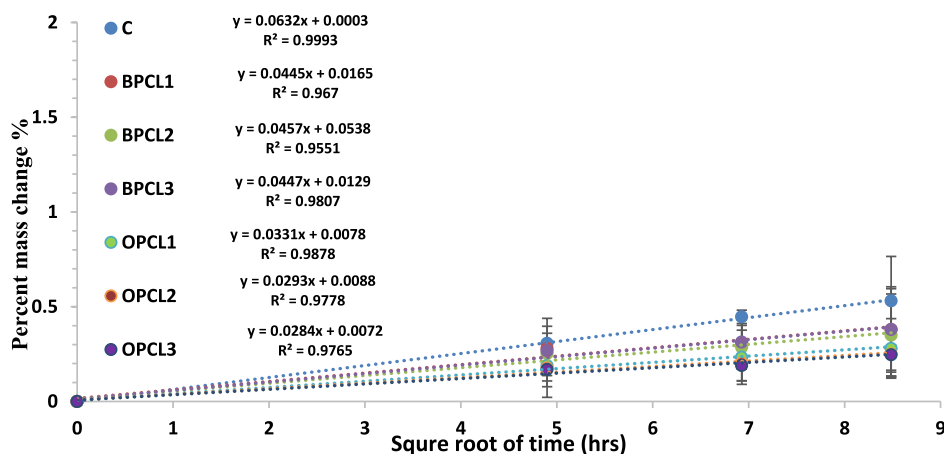


Figure 13. Initial gradient of mass (m_i) plotted against the square root of time (wt %/h 0.5), measured at 24, 48, and 72 h for all the control and experimental groups.

1. After 1 week, the rate of mass change increased for all the groups, while the control exhibited the highest value. From week 1 to week 6 all groups continued to absorb water at slower rates.

Figure 13 shows the initial gradient of mass (m_i) measured at 24, 48, and 72 h and plotted against the square root of time. The lowest initial mass gradient was recorded for OPCL 3,

while the control group exhibited the greatest initial mass gradient.

Figure 14 shows the final gradient of mass (m_f) plotted against the square root of time (wt %/h 0.5) and measured from week 1 to week 6. The greatest mass change at week 6 was measured for the control group.

The soft lining material is constantly present in a moist environment; therefore, an ideal soft lining material should be

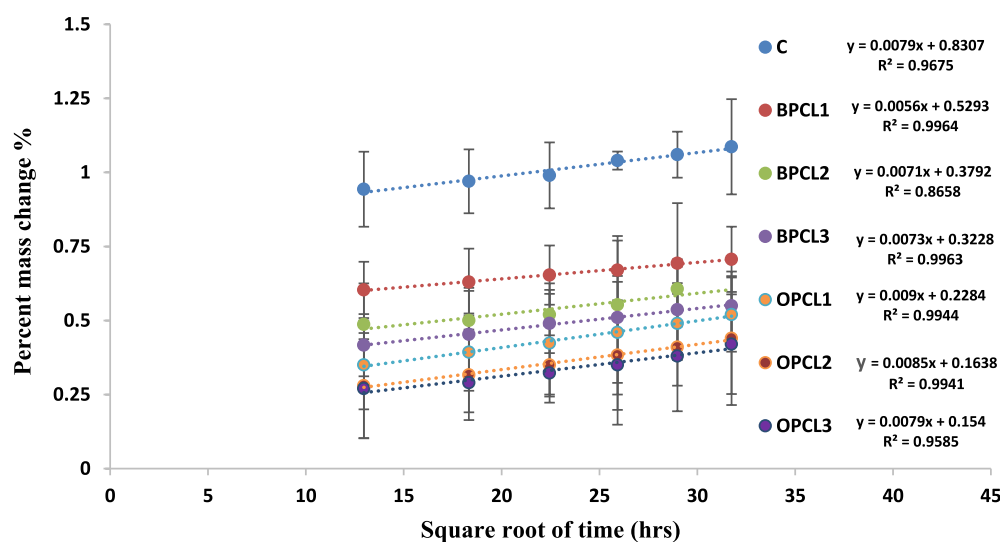


Figure 14. Final gradient of mass (m_t) against the square root of time (wt %/h 0.5), measured from week 1 to week 6 for the control and experimental groups.

dimensionally stable and should neither be soluble in oral fluids nor should it uptake water from its environment.⁶ Compared with acrylic soft denture liners, silicone denture liners exhibit less percent mass change due to the absence of leachable plasticizers.³⁵ According to ISO 10139-2,¹⁸ the percent mass change of a soft liner sample should not be more than 2%. In this study, the mean percent mass change after all intervals was greater for control groups, followed by BPCL groups, and the least mass change was observed in groups that contained OPCL. After 1 week in distilled water, % of mass change for the control group was 0.94%, which is in agreement with the results of previous studies.^{36,37} The decrease in mass change of experimental groups is due to the presence of an alkyl group in the structure of ionic liquids. The length of the alkyl chain influences the hydrophobicity of the ionic liquid;³⁸ hence, the OPCL groups that had longer chain lengths showed the least percent mass change. The initial and final gradient of mass depicts an almost linear uptake of water by all the groups, as shown in Figures 12 and 13, respectively. The studies conducted on percent mass change in different media suggest that the highest percentage of mass change in silicone soft liners occurs in distilled water compared with other solutions.³⁷ One-way ANOVA showed a statistically non-significant difference (p -value > 0.05) between groups after day 1, but a significant difference (p -value < 0.05) was found between groups after week 1 and week 6.

3.4. Antifungal Testing. **3.4.1. Disc Diffusion and Agar Well Diffusion.** In the disc diffusion method, no zones of inhibition were observed for the experimental and control groups. In the agar well diffusion method, the ionic liquids (BPCL and OPCL) diluted in different concentrations (0.65–10%) in DMSO were poured into the wells. Zones of inhibition were shown against *Candida albicans*, as shown in Figure 15.

The lowest zones of fungal inhibition were found in group BPCL 1 (10.2 ± 0.08 mm) and the highest in group OPCL 3 (20.5 ± 0.12 mm); the details are shown in Figure 16. There was a statistically significant ($p \leq 0.05$) difference found between all groups except OPCL1 and BPCL3.

3.4.2. Minimum Inhibitory Concentration. After 24 h, the 96-well plates were checked for turbidity to determine the

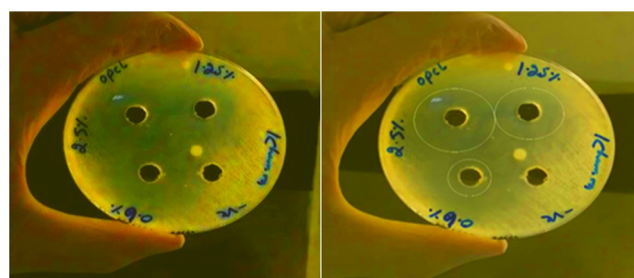


Figure 15. Zones of inhibition around wells.

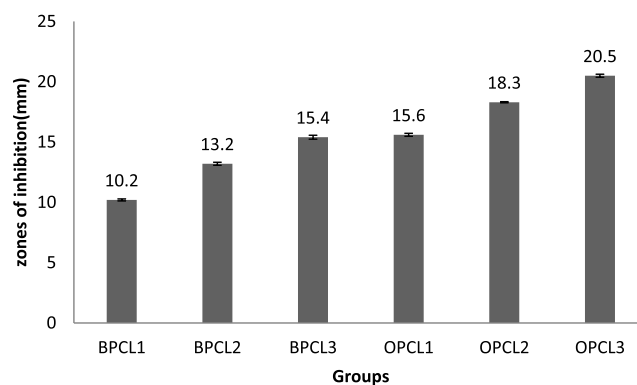


Figure 16. Zones of inhibition (mm) with error bars (SD) for control and experimental groups.

MIC of the ionic liquids, as shown in Figure 5. As our compound was colored, giving it a milky appearance, instead of using the ELISA reading method, we followed a visual plate assay for confirmatory purposes. The Muller Hinton agar plate was observed to check the growth. In the negative control group, no visible colonies were seen. While in positive control groups, the visible colonies of *Candida albicans* were seen, as shown in Figure 17.

There was no growth observed up to the 11th concentration for BPCL and up to the 10th concentration for OPCL. Growth was observed on further dilutions. So, it was concluded that for BPCL, $9.7 \mu\text{g/mL}$ was the MIC, and for octyl pyridinium chloride, $4.8 \mu\text{g/mL}$ was the MIC, as tabulated in Table 3.

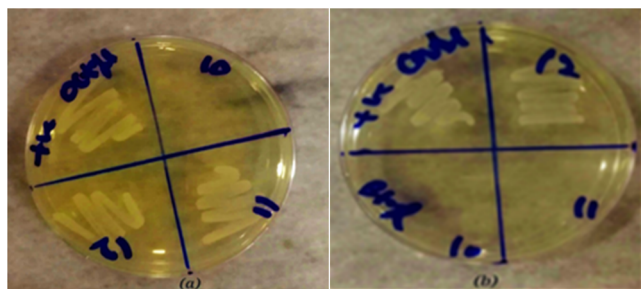


Figure 17. Visual plate assay with visible candida growth.

Table 3. MIC of the Molten Salts

s.no	BPCL salt conc. (%)	growth	OPCL (%)	OPCL
1	10	no growth	2.5	no growth
2	5	no growth	1.25	no growth
3	2.5	no growth	0.65	no growth
4	1.25	no growth	0.325	no growth
5	0.65	no growth	0.1625	no growth
6	0.325	no growth	0.081	no growth
7	0.1625	no growth	0.040	no growth
8	0.08125	no growth	0.020	no growth
9	0.04062	no growth	0.010	no growth
10	0.020	no growth	0.005	no growth
11	0.0101	no growth	0.002	growth
12	0.005	growth	0.001	growth
13	0.002	growth	0.0005	growth
14	positive control	growth	positive control	growth

Candida albicans, which is a major contributing factor in the development of denture induced stomatitis, is a part of normal flora. Under favorable conditions, the adherence of these pathogens is increased and causes candidiasis.¹⁹ Microbial colonization by *Candida albicans* is a major drawback of silicone soft denture liners;³⁹ therefore, we evaluated the antifungal properties of ionic liquid-modified silicone through disc diffusion and well diffusion methods. The minimum fungicidal concentration was also determined. For all the control and experimental groups, no zones of inhibition were noted on the disk diffusion method. The diffusion method was performed to determine the antifungal potency of the BPCL and OPCL molten salts. Comparing both salts, the maximum zones of inhibition were obtained for 2.5% of OPCL, as shown in Figure 10. The antifungal activity of these molten salts, according to previous studies,^{12,40} is associated with the ionic activity of the ionic liquids, which causes a decrease in the ergosterol content and disruption of cellular processes in fungi that lead to their growth inhibition. A study also reported that ionic liquids with greater alkyl chain length have more antifungal potency, which is also confirmed by our study, as octyl salts showed greater zones of inhibition.⁴¹ Previously, ionic liquid has been added to short-term soft denture liners with success, and a noticeable decrease in the colony count of *Candida albicans* was observed.¹⁹ The MIC results when compared with other alkyl pyridinium ionic liquids show that it is effective in minimal amounts⁴⁰ and also confirm that the octyl pyridinium ionic liquids are more potent antifungal agents compared to BPCL.

3.5. Biocompatibility. Figure 18 shows the cell viability assay of culture cells and Molloplast-B (control), and their composites with 0.6 and 1.25% octylpyridinium chloride and 2.5, 5, and 10% BPCL ionic liquids. The addition of both ionic

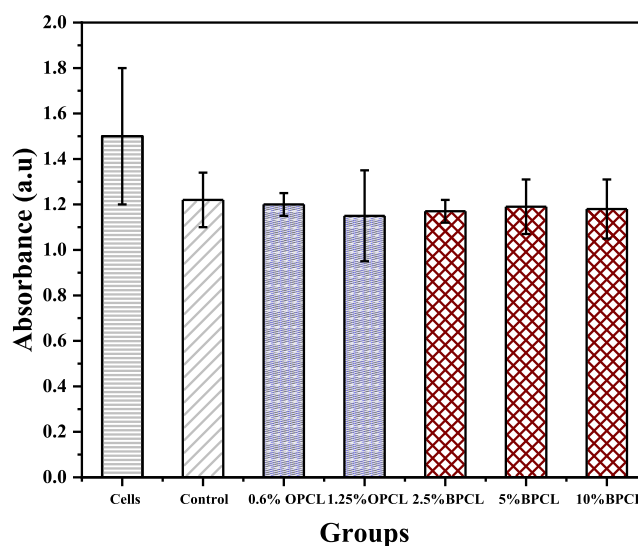


Figure 18. Cell viability assays of different composites.

liquids in Molloplast-B has no significant effect on the cell viability concerning control as the absorbance values are in the same range. However, with respect to cell culture, the control and their composite showed lower cell viability. The types of cation ionic liquids due to their chemistry relate differently to the cells Wang et al.⁴² observed that the imidazolium-based ILs showed higher toxicity against HeLa cells than pyridinium and ammonium-based ILs with the bromide anion trend $[N4222]^+ < [C4py]^+ < [C4C1im]^+$. In our study, pyridinium-based ionic liquids with moderate toxic effects were used. Generally, ionic liquids with longer alkyl chains on the cation moiety are considered toxic as compared to those with short alkyl chains due to lipophilic properties. This lipophilic property is owed to their interaction with the phospholipid bilayers of biological membranes, disrupting the cells.⁴³ In our study, two alkyl chain lengths, octyl and butyl, with pyridinium chloride were studied. The addition of both types of ionic liquids in Molloplast-B has no toxic effect, which might be related to their lower concentrations (0.6–10%) as well as the slow release pattern from composite. Similarly, no toxic effect was observed for 1-*n*-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide (BMI.NTf₂)-based microcapsules loaded (2.5, 5, or 10 wt %) in dental resin, and the cell viability was more than 90% for human keratinocytes.⁴⁴ Moreover, Garcia et al. also observed no changes in the pulp cells' viability for zinc-based particles with 1-*n*-butyl-3-methylimidazolium chloride ionic liquid (1, 2.5, or 5 wt %) addition in the dental adhesive.⁴⁵

4. CONCLUSIONS

The ionic liquids were successfully mixed with the commercial silicone denture lining material, which improved their various properties. The addition of OPCL and BPCL improved the surface homogeneity, which would reduce the attachment of microbes. The TBS is measured in the range mentioned by the ISO standard 0.44 MPa. All experimental groups showed a decrease in bond strength with an increase in the addition of ionic liquids. The shore A hardness that is related to the softness of the material was observed to increase with the addition of BPCL. While there was a decreasing trend observed for OPCL. The results are in line with ISO 10139-2, i.e., ≤ 50 shore units. The least percent mass change was observed in OPCL groups, followed by BPCL groups and the

control. The OPCL showed more potent antifungal activity against *Candida albicans* compared with BPCL. The addition of both ionic liquids to Molloplast-B has no significant effect on the cell viability.

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Notes

The authors declare no competing financial interest.

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