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CHEMICAL PROPERTIES AND BIOCOMPATIBILITY OF MICROCRYSTALLINE CELLULOSE REINFORCED DENTURE BASE RESIN MATERIAL: AN *In Vitro* STUDY

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ABSTRACT

This study aimed to determine the chemical functional groups and cytotoxicity level of denture base resin (DBR) material reinforced with oil palm based microcrystalline cellulose (MCC) at different concentrations. Three MCC-reinforced polymethyl methacrylate (PMMA) specimens were compared with conventional and commercially available high-impact PMMA. The test groups were represented by adding various MCC and acrylic polymer concentrations. Fourier transform infrared spectroscopy (FTIR) was conducted to compare the chemical structure of the specimen groups. Cytotoxicity was evaluated by filter diffusion test. Extracts from study groups were tested using the MTT assay protocol for cell viability and proliferation with normal human oral fibroblast (NHOF). The FTIR analysis showed that good bonding between MCC-OPEFB and PMMA as the functional group strength was reliable in cellulose-treated PMMA. The analysis also confirmed high integrity and successful grafting between the two. Cell viability assays showed that exposure of NHOF to polymer-MCC mixture eluates did not promote cell death or considerable toxic impacts. Suitably processed oil palm-based MCC-reinforced DBR can improve the bonding and integrity of the composite material without compromising its biocompatibility leading to the development of reinforced DBR with enhanced microstructural and chemical properties.

Keywords: cytotoxicity, FTIR, microcrystalline cellulose, oil palm empty fruit bunch, polymethyl methacrylate.

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INTRODUCTION

Polymethyl methacrylate (PMMA) is widely used for removable complete and partial dentures due to its aesthetic qualities, biocompatibility and clinical performance (Im et al., 2017). However, its mechanical properties, specifically its low flexural (Karci et al., 2019) and impact (Mowade et al., 2012) properties, have prompted researchers to explore improving its physical characteristics. One approach is to add natural or synthetic additives, which have been shown to enhance the material's strength and durability (Gad et al., 2017; Soygun et al., 2013).

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Microcrystalline cellulose (MCC), an extracted compound, is deemed one of the richest yielded renewable organic materials (Seddiqi et al., 2021). MCC of the oil palm biomass is utilised to prepare environmentally friendly polymer composites (Soom et al., 2009), with its strengthening function and low density, increases the mechanical properties of the polymers and has appropriate aesthetic values. In addition, MCC has also been reported to enhance polymer composites' thermal stability, barrier properties, and biodegradability (Lang et al., 2022). MCC powder was homogeneously mixed with the PMMA polymer and distributed evenly in the resin dough mixture, despite its insufficient impregnation of fortifying particles like fibres, rods, or flakes within the polymer matrix due to its high polymer/monomer mixture consistency. Studies have reported that PMMA reinforced with MCC oil palm empty fruit bunch (OPEFB) fibre has better flexural strength and modulus compared to conventional and commercially modified PMMA resins (John et al., 2014).

Despite being the material of choice, some denture wearers have reported irritation and allergic reactions induced by the synthetic structure of the acrylic utilised as a denture base when in contact with the oral mucosa, primarily the unreached methyl methacrylate (MMA) residuals (Gautam et al., 2012). The incomplete conversion of MMA monomer to its polymer in the polymeric matrix will affect the properties of the final product. Besides, other toxic agents, such as methacrylic acid and formaldehyde residue, remain within the PMMA denture base after processing (Alqutaibi et al., 2023). One of the measures for biocompatibility is that the material is not toxic to cells (Kohli & Bhatia, 2013). *In vitro* cytotoxicity assays are necessary to screen any newly developed materials before clinical application. The aim of this study was to evaluate PMMA denture base resin biocompatibility when mixed with MCC in different weight percentages and its molecular composition and structure.

MATERIALS AND METHODS

Table 1 shows the commercial details of the conventional, high-impact acrylic and synthesised OPEFB based MCC materials.

Cytotoxicity Testing

Preparation of specimens. A total of five groups of denture base resin (DBR) test specimens (n = 3), measuring 65 x 10 x 3 mm were produced according to International Organization for Standardization (ISO) specifications (ISO, 2013). Samples of groups A and B (*Table 2*) were prepared with the powder-to-liquid ratio recommended by the manufacturer. Both PMMA and MCC powders were weighed according to *Table 2* using a digital weighing device (Sartorius-AX224, Sartorius AG, Germany) at an accuracy of ± 0.01 mg during specimen preparation for groups C, D and E. The specimens were prepared by slicing segments of the previous rectangular test pieces with a diamond cutting disc and made into cylindrical specimens with a measurement of 5.0 ± 0.2 mm diameter and 2.5 ± 0.2 mm thickness.

Cell Culture

Thawing of cells. Cryovials containing frozen normal human oral fibroblast (NHOF) were transported from liquid nitrogen to a water bath at room temperature. During the thawing, the vial was kept on the surface of the water bath by applying an intermittent light pressure. The cells were thawed and processed within a few seconds to ensure viability and relocated to a T-75 culture flask containing 30 mL of growth medium, then incubated at 37°C, 5% CO_2 at a flat position overnight. The medium was repeatedly replaced till the cell monolayers became confluent.

Specimen preparation for MTT assay. A total of 15 acrylic specimens of the five groups (n = 3) were placed in test tubes with Dulbecco's Modified Eagle's Medium (DMEM). An additional test tube was packed with only the medium and cells as a control. All test tubes were placed in a water bath at 37°C for 24 hr, then the acrylic specimens were removed gently from the test tubes and the NHOF extract was filtered using a sterile syringe filter of 0.22 µm. The NHOF were grown with 5% CO_2 at 37°C in the DMEM and 10% fetal bovine serum (FBS) containing penicillin/streptomycin and amphotericin-B. The cells were plated in 24-well tissue culture trays (104 cells/cm) for a 24-hr incubation period. After this period, the

TABLE 1. RELEVANT COMMERCIAL DETAILS OF THE MATERIALS

Material	Product information	Manufacturer
Acron Duo	Heat-cure denture base acrylic	Kemdent Works, United Kingdom
Acron HI	Heat-cure high impact acrylic	Kemdent Works, United Kingdom
MCC of OPEFB	Microcrystalline cellulose from oil palm empty fruit bunch.	Malaysian Palm Oil Board (MPOB), Malaysia

TABLE 2. MASS COMPOSITION OF THE SPECIMENS

	Test material	Powder (g)	Liquid (mL)	MCC (g)
A	Heat-cure denture base acrylic (conventional)	12.00	4.00	Nil
B	Heat-cure high impact acrylic	12.00	4.00	Nil
C	Heat-cure denture base acrylic (conventional) with 2% cellulose (2% of monomer liquid increased)	12.00	4.25	0.24
D	Heat-cure denture base acrylic (conventional) with 2% cellulose (2% acrylic powder reduced)	11.76	4.00	0.24
E	Heat-cure denture base acrylic (conventional) with 5% cellulose (5% acrylic powder reduced)	11.40	4.00	0.60

Note: MCC - microcrystalline cellulose.

cultures in the wells were subjected to cytotoxicity, and assessment was completed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. Cytotoxicity was assessed based on cell viability. A percentage of cell viability greater than 80% is considered non-cytotoxic; 80%–60% weak; 60%–40% moderately and less than 40% strongly cytotoxic (López-García et al., 2014).

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

All specimens from Group A, B, C, D and E used for a previous study (Rahaman Ali et al., 2020) were prepared by grinding one cured acrylic specimen from every group using a diamond bur. Before the FTIR testing, each ground specimen was carefully preserved in a dry, sterile and well secured container. The potassium bromide (KBr) method was used for the FTIR analysis. This involved mixing the ground acrylic with KBr in a ratio of 1:200 and compacting the mixture into pellets in a stainless-steel die.

Transmission spectra were sourced from a FTIR spectrometer with a spectral resolution of (SPECTRUM 400, Perkin Elmer, UK) and a wavelength accuracy of 650–4,000 cm^{-1}. Specimens from each group were placed in the window, and analysis was conducted by running the spectrum. The point of substantial transmittance peaks was established with the SPECTRUM™ 10 STD software (Perkin Elmer, USA).

RESULTS AND DISCUSSION

Results showed that all test groups were classified as non-cytotoxic, with no statistically significant difference among all groups; the viability of all groups was more than 90% (*Figure 1*). Among the five groups, the commercially reinforced specimens (Group B) showed the lowest cell viability (91.7%). In contrast, Group E specimens showed the highest cell viability (99.2%).

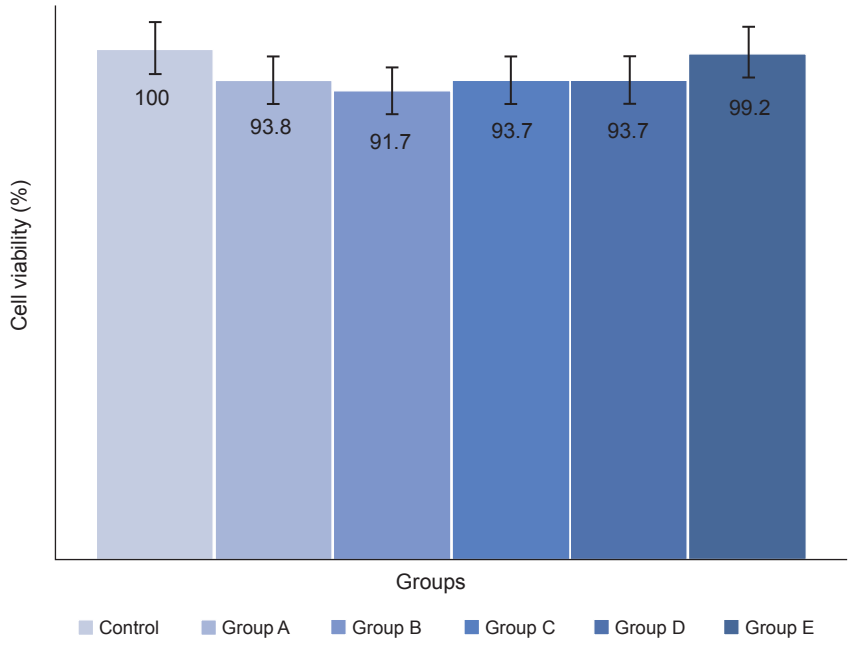

Figure 1. MTT test reporting on the cell viability of the specimens.

The FTIR spectrum for MCC from EFB (*Figure 2*) exhibited two main absorbance regions namely the functional group region (4,000–1,300 cm^{-1}) and the fingerprint region (1,300–400 cm^{-1}). The concentration of the peak at 894.11 cm^{-1}, associated with the β-glycosidic linkages, declined as the crystallinity of the samples intensified. The hydrolysis process affected the cleavage of glycosidic linkages of cellulose to produce MCC. The structural property of MCC was also considered by applying X-ray diffraction (XRD), and the outcome demonstrated that it has a crystallinity index of 72% (Fatiha et al., 2021). With a high crystallinity index, MCC is considered suitable for green bio composite production (Ramli et al., 2015). A high crystallinity index in MCC can significantly enhance the material's mechanical strength, rigidity and thermal stability. The ordered and tightly packed crystalline regions contribute to these improved properties, making the bio composite more durable and resistant to mechanical stress. Additionally, high crystallinity reduces moisture absorption, which is crucial for applications where dimensional stability and resistance to environmental factors are essential. This crystallinity also improves the compatibility between MCC and the polymer matrix, ensuring better dispersion and stronger interfacial bonding within the composite. These attributes make high-crystallinity MCC particularly suitable for producing bio composites used in demanding applications that require strength, stability and durability.

Figure 3 summarises the FTIR spectra of the PMMA specimens with and without MCC reinforced. The prominent bands in the zone ranging from 3,400–3,500 cm^{-1} are associated with OH bending, while the bands between 2,800–2,900 cm^{-1} are responding to –CH aliphatic compounds. The bands at 1,435–1,438 cm^{-1} represent $–CH_2–$ group bending. The penetrability at 1,141–1,158 cm^{-1} is relevant to the C–O–C group. It can be observed that all specimens show similar bands in the 1,000–1,800 cm^{-1} range. However, there are some differences in the intensity of the bands. The specimens modified with MCC show an additional band around 3,300 cm^{-1}, assigned to the hydroxyl (–OH) group of cellulose. This band is more prominent in specimens C, D and E, indicating that adding MCC increases the amount of –OH groups in the composite. In addition, specimens C, D and E show a decrease in the intensity of the bands between 1,720–1,750 cm^{-1}, which are related to the carbonyl (C=O) stretching vibration of PMMA. It suggests that MCC reduces the amount of C=O bonds in the composite. Adding MCC to PMMA modifies the chemical structure of the composite by introducing –OH groups and reducing the amount of C=O bonds. When MCC is introduced into PMMA, the composite undergoes significant chemical and structural changes. MCC is rich in –OH groups, which can interact with the C=O groups of PMMA through hydrogen bonding. This interaction weakens the C=O bonds, decreasing the intensity of the C=O stretching vibrations observed in the FTIR spectra. Additionally, the hydroxyl groups from MCC contribute directly to an increase in the –OH group content in the composite, as reflected by the more prominent –OH stretching bands. There may also be some chemical modification, where the hydroxyl groups from MCC react with the ester groups in PMMA, further reducing the number of carbonyl groups. These interactions and the potential disruption of PMMA's crystalline structure result in a composite material with increased –OH groups and a reduced presence of C=O groups.

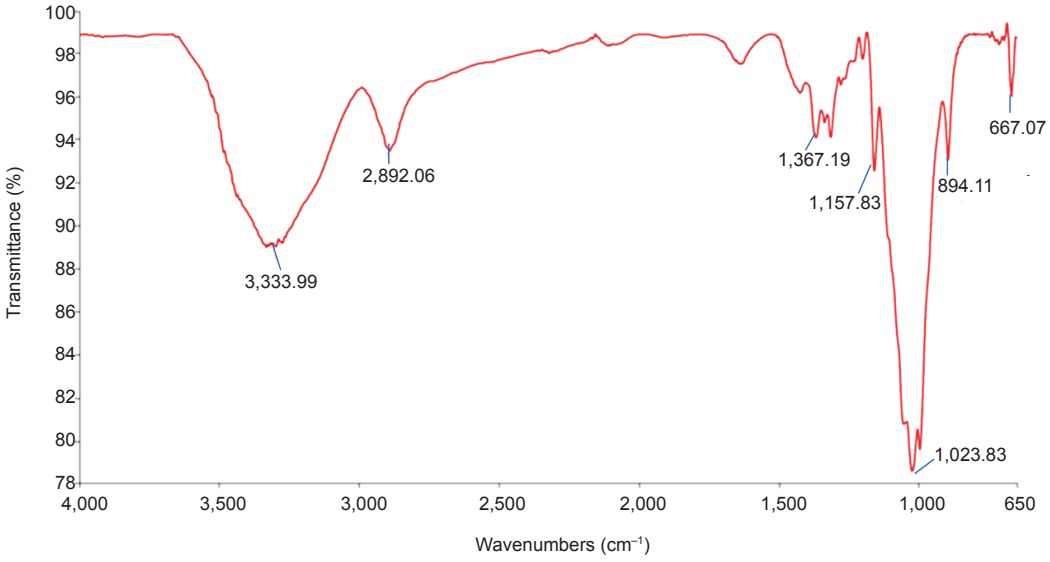

Figure 2. FTIR spectrum of MCC from empty fruit bunch cellulose.

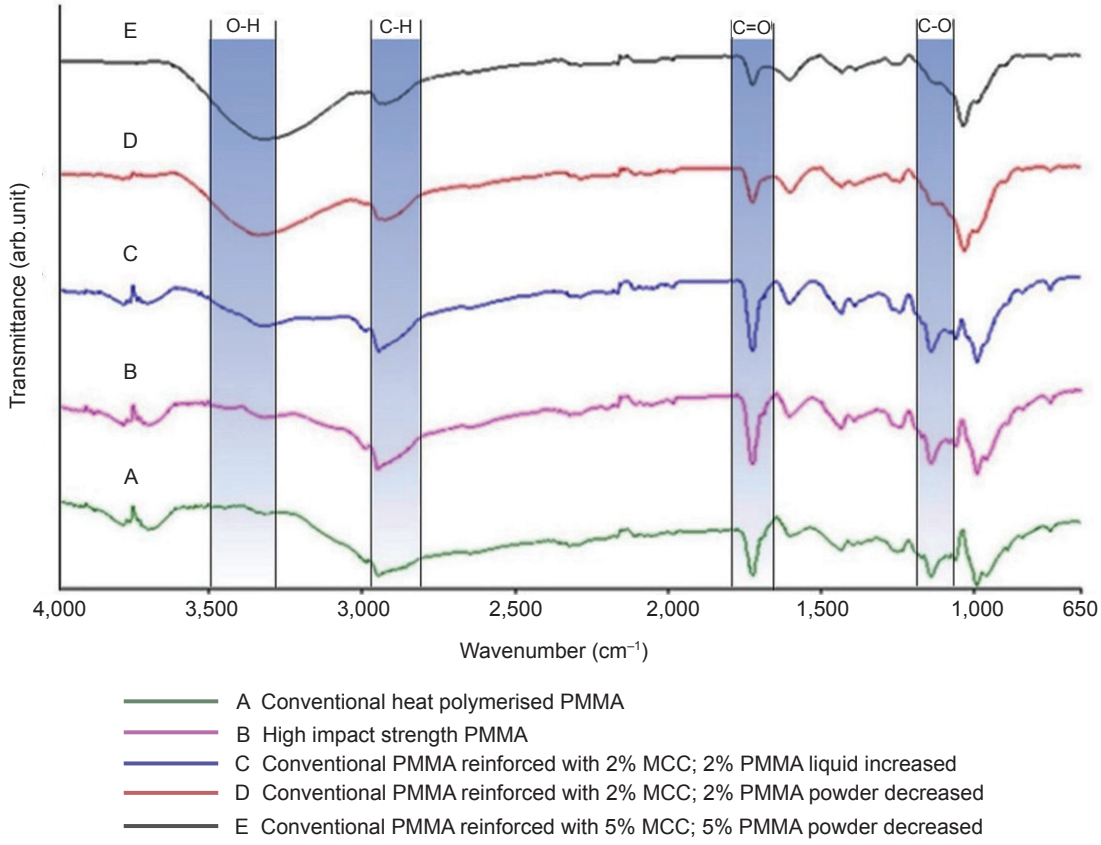

Figure 3. Overlay of the FTIR spectra of the PMMA and MCC specimens.

Developing dental materials with desirable biological and toxicological properties is essential for clinical use (Kulak-Ozkan et al., 2003). A critical step in the testing procedure is the evaluation of *in vitro* cytotoxicity, which helps to determine how cells react when in contact with the material and the process of cell death. The capacity to produce the desired tissue-biomaterial interfacial reaction is critical for the future of biomaterials science (Ratner et al., 2020).

This study used human fibroblast cells as a model for denture bases in direct contact with oral mucosa. The results showed that all test groups were non-cytotoxic, with more than 90% viability. The high cell viability observed in all groups suggests that these materials can be safely used in clinical applications without causing any harmful effects on surrounding tissues or cells. It is vital to consider that although the cytotoxicity of these materials was evaluated *in vitro*, additional studies are necessary to evaluate their biocompatibility *in vivo*. *In vivo* studies are essential to fully understand the biological response to dental materials, as they consider factors such as the immune response, tissue integration and long-term effects.

The main causative factor of denture sore mouth is the hypersensitive response of oral tissue to denture base material. *In vitro* studies have reported that residual monomer can be released from the denture into the oral cavity (Leggat & Kadjarune, 2003; Rashid et al., 2015). The filler in the cured denture resin matrix releases the unreacted residual monomer into the oral cavity causing clinical signs and symptoms of hypersensitivity. This chemo toxic irritant effect can be eliminated by ensuring a complete curing process and storing processed dentures in water for at least one day before use, preferably at 37°C (Bayraktar et al., 2005). In another study (Raszewski et al., 2021), it was reported that bioactive glass fillers were added to PMMA for the release of fluoride ions to inhibit microbial colonisation and the formation of plaque in the oral cavity of the denture wearer. Therefore, it is important to determine the biocompatibility when introducing a new agent into a dental product. The current study confirms that adding MCC-OPEFB to the PMMA acrylic denture resin does not compromise its biocompatibility and cytotoxicity level.

Conversely, the FTIR spectra of PMMA specimens modified with MCC provide valuable insights into the molecular structure and properties of the resulting composites. The spectra reveal distinct bands and variations in their intensities, depending on the concentration of MCC and the type of PMMA used. One of the spectra's most notable observations is the region's changes

associated with –OH and C–O–C groups. The presence of added bands at 3,318 and 3,418 cm^{-1} in the MCC-reinforced groups indicates the occurrence of –OH (H-bonded) groups, likely derived from the cellulose component of MCC. Additionally, an extra band at 1,641 cm^{-1} in the MCC groups indicates the presence of –OH groups associated with cellulose. These changes suggest potential improvements in the bond between the PMMA matrix and the filler particles, leading to improved mechanical properties. MCC has a unique character because it can be grafted with other polymers through the hydroxyl group, as reported by Hubbe et al. (2008). The various wavelengths in the FTIR spectra can provide valuable insights into the changes that occur when an additive is introduced into a polymer matrix. For instance, changes in spectral bands' shape or relative intensities indicate that the additive has modified the original compound (Larkin, 2018). Regarding C=O and C–O bands, PMMA reinforced with MCC showed variations in their intensities depending on the concentration of MCC and the type of PMMA used. The greatest concentration of the C=O band was realised in the high-impact acrylic PMMA (Group B), followed by conventional PMMA reinforced with 2% MCC (Group C) and conventional PMMA (Group A). Meanwhile, the C–O band intensity increased with increasing MCC concentration. The cellulose-containing groups showed a stronger intensity of the functional group, which is a positive indication from a chemical standpoint (Tjeerdsma & Militz, 2005).

The changes in the spectral bands observed in the MCC-reinforced groups may have implications on the features of the resulting PMMA composite. The increased presence of –OH groups may enhance the bond between the PMMA matrix and the filler particles, improving mechanical properties. The changes in the C–O–C groups may also have implications for the water sorption and solubility of the composite, which are essential considerations in dental applications. It is worth noting that in Groups C and D, which were reinforced with 2% MCC, there was a lowering of the intensity of the bands at 1,721 and 1,641 cm^{-1}. It may indicate that the adding of MCC has some effect on the crosslinking mechanism of PMMA, which could affect the overall strength and stability of the material. In addition to the abovementioned changes, the spectra of PMMA reinforced with MCC also showed variations in the 1,300–1,000 cm^{-1} region. The band at 1,158 cm^{-1}, which is associated with the C–O–C group, showed an increase in intensity in the specimens with the addition of MCC (Groups C, D and E) compared to the conventional PMMA (Group A) and high-impact PMMA (Group B). This could be ascribed to the presence of cellulose in the MCC, which could enhance the intermolecular interactions between MCC and PMMA.

The FTIR results in this study provide valuable insights into the changes in the molecular structure of PMMA modified with MCC. The spectra obtained from different PMMA specimens reinforced with different concentrations of MCC reveal distinct bands and variations in their intensities. These changes indicate the potential for the composite material to form crosslinks between MCC and PMMA, which can enhance the overall strength and stability of the material, making it a promising option for use in prosthodontics and other dental applications. The changes observed in the FTIR spectra, specifically the increase in –OH groups and the reduction in C=O groups due to the introduction of MCC into PMMA, could have a correlation with the cell viability results. The increase in hydroxyl groups generally enhances the hydrophilicity of the material, which can improve cell attachment and proliferation. This is because cells tend to favor hydrophilic surfaces for adhesion, which can promote better integration and viability. On the other hand, reducing carbonyl groups might reduce the potential cytotoxicity associated with the ester components in PMMA, further supporting a more favorable environment for cell growth. Recent *in vitro* simulation by the authors of this study have demonstrated that incorporating MCC into acrylic resin can significantly enhance the denture flexural strength and modulus, even after thermal cycling (Rahaman Ali et al., 2020). The scanning electron microscope (SEM) result showed that the resin treated with various concentrations of OPEFB-MCC displayed no micro-gaps between the filler particles and the acrylic resin matrix. These results suggest that using MCC as a reinforcing agent can potentially improve denture longevity and prevent denture fractures, which is a significant concern for many denture wearers. Moreover, the ease of adding, blending and handling MCC alongside PMMA resin further supports its possible clinical application. The findings of the current study provide a promising avenue for developing new dental materials with improved mechanical properties, which can significantly impact the oral health and quality of life of denture wearers.

Overall, the use of natural agents that are environmentally friendly, from a renewable OPEFB-MCC to reinforce acrylic denture resin, is a promising method to improve the biological and toxicological functions of the existing commercially available synthetic reinforced PMMA resins. Cytotoxicity tests conducted in this study demonstrated that the natural agent used did not adversely affect human fibroblast cells. However, more *in vivo* studies are necessary to evaluate the clinical performance of these materials. Additionally, adding MCC to conventional PMMA may lead to changes in the composite's molecular structure, affecting its properties and performance in dental materials. These findings suggest that

MCC can be used in other dental applications, and additional studies are necessary to investigate this possibility.

CONCLUSION

The results of this study demonstrated that the addition of MCC to PMMA resin resulted in a copolymer structure, which improved the microstructural and chemical properties of the PMMA resin. The FTIR changes indicating increased –OH and reduced C=O groups likely contribute to improved cell viability, as these modifications result in a more biocompatible surface and conducive to cell viability. The biocompatibility tests confirmed that the resulting composite had an acceptable biological response, making it a promising material for a wider dental application. Furthermore, the outcomes of this study suggest that MCC has the potential to improve the clinical service life of dentures. The research findings presented in this study also have the potential to extend the application of OPEFB-MCC to other dental materials, paving the way for future research into the potential applications of this natural agent in dentistry to promote sustainable use of terrestrial ecosystem which is one of the pillars of the 2030 agenda for the United Nations' Sustainable Developmental Goals.

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