*Available online*www.ijpras.com

International Journal of Pharmaceutical Research&Allied Sciences, 2018, 7(2):111-118

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

The Use of Fourier Transform Infra-Red (FTIR) Spectroscopic Analysis and Cell Viability Assay to Assess Pre-polymerized CAD\CAM Acrylic Resin Denture Base Materials

Abdulelah M. Bin Mahfooz*, Manal R. Alammari

Department of Oral and Maxillofacial Prosthodontics, King Abdulaziz University, University Dental Hospital, Jeddah, Kingdom of Saudi Arabia
**Corresponding Author*

ABSTRACT

Background: Polymethyl methacrylate (PMMA) resin is one of the widely used industrial polymeric materials in prosthodontics. At present with the progress of technology, computer-aided design and computer-aided manufacturing (CAD/CAM) is successfully employed on a great scale in prosthodontics. The current research evaluated in vitro the chemical composition of CAD/CAM as denture base material by using Fourier Transform Infrared (FTIR) spectroscopic quantitative analysis and likewise the biocompatibility for clinical usage. Materials and methods: Forty specimens were prepared and divided into two groups of 20 each, group A was made of heat polymerized PMMA, while group B was fabricated from CAD/CAM blocks. The test specimens of the groups (A and B) were subdivided into 2 subgroups, each of 10 (A1, A2 and B1, B2). The subgroup test specimens of (A1, B1 and A2, B2) were prepared with standardized disc shape of dimensions (25mm diameter and 3mm thickness) to evaluate the chemical composition by using (FTIR) and the cytotoxicity by cell viability and live/dead staining. Results: The infra-red spectra analysis of subgroup (B1) revealed that there was a three-transmission peak and there was a significant change observed between subgroups (B1 and A1) that concluded there is an internal difference in the structure of both subgroups. The survival cell rate percentage of hMSCs of subgroup (A2, B2) and -ve control group after 24hrs of incubation period represented no statistical difference. Live cells observed in the control as well as the treatment subgroups (A2and B2) with retained polyanionic dye calcein, presented an intense green fluorescence with no morphological changes and no evidence of increasing cell death because the number of cells emitting red fluorescence that penetrate the cell membrane by EthD-1 was not altered. Conclusion: FTIR spectroscopy analysis indicated the presence of very small amount of residual monomer in CAD/CAM denture base material that was nearly at the same level of heat polymerized type with no significant difference. To detect the cytotoxic effect of residual monomer, two sensitive methods of cell viability were used to distinguish the biocompatibility. Both tests revealed that the tested groups displayed no cytotoxic effect on the viability of hMSCs after 24hrs incubation period.

Keywords: *Acrylic resin denture base, CAD/CAM denture base, Cell viability, Cytotoxicity assay, FTIR spectroscopy analysis, PMMA*

INTRODUCTION

Polymethyl methacrylate (PMMA) resin is one of the widely used industrial polymeric materials not only in dentistry but also in biomaterials. It is used in the construction of denture bases, owing to their worthy aesthetics, easy processing, and comparative simplicity of repair [1, 2]. However, inadequate mechanical properties render this material with some limitation on applications [3].

In spite of the improvements in the physical properties of PMMA over the years, they were subjected to certain criticism such as volumetric shrinkage. The presence of residual methyl methacrylate monomer and low impact strength which allow acrylic resin dentures to fracture [4,5]. PMMA denture base takes up saliva and water, which

move slowly into tiny pores inside the denture prompting reduction in mechanical properties of the material thus increasing the surface roughness and producing an unpleasant odor [6].

At present with the progress of technology, computer-aided design and computer-aided manufacturing (CAD/CAM) are successfully employed on a great scale in dentistry. It has been used for the fabrication of inlays, crowns, fixed partial dentures, implant abutments/structure and maxillofacial prostheses. CAD/CAM system lately has become commercially obtainable for fabrication of complete dentures and is considered as an alternative to conventionally processed acrylic resin denture bases [7].

CAD/CAM denture system is an innovative method for construction of removable prostheses. It is composed of blocks of pre-polymerized denture base material supplied as resin block for milling of denture base. It eliminates the need for the wax; flasks boil tanks, packing presses and heat processing units required for conventional denture construction. The fabrication of the denture base by CAD/CAM provides superior fit and strength when compared to conventionally processed bases. Moreover, it undergoes no polymerization shrinkage, contains less residual monomer and is more hydrophobic [8, 9].

Variations in the components, structure and the clarity level of the existing resins in the market, the monomer conversion rate and manipulative variables may affect the toxicity of the resins [10, 11]. In general, polymeric CAD/CAM materials contain a polymeric matrix that is reinforced by inorganic, organic or composite fillers. CAD/CAM resin blocks are industrially polymerized under standardized parameters at high temperature and pressure to guarantee that the microstructure and the mechanical properties of the resin blocks display continuous excellence. Its industry is based on renewable resources [12, 13, 14].

Quantitative analysis of CAD/CAM material has been the topic of many studies. Infrared (IR) radiation is a noninvasive and nondestructive type of radiation and it causes the vibration of the covalent bonds of molecules when absorbed. Fourier transform infrared (FTIR) spectroscopy is a widely-used and preferred method of IR spectroscopy due to its speed and sensitivity [15]. Although biological safety of CAD/CAM material from various manufactures has been approved for clinical usage, there is a little information given on the chemical composition of CAD/CAM as denture base material system that is kept undisclosed. Therefore, the aim of the present study was to evaluate in vitro the chemical composition of CAD/CAM as denture base material by using Fourier Transform Infrared (FTIR) spectroscopic quantitative analysis and likewise to evaluate the biocompatibility of this material for clinical usage.

MATERIALS AND METHODS

Preparation of the specimens

Forty specimens of two different denture base materials were prepared in this study. The denture base materials used in this study were heat polymerized acrylic resin (group A included 20 specimens, PMMA, vertex RS Dentimex Netherlands) and cadlcam acrylic resin material (group B included 20 specimens, Polidentd.o.o. VolčjaDraga 42, Sl-5293 VolčjaDraga, Slovenia), and the test specimens of groups (A and B) were subdivided into two, each of 10 (A1, A2 and B1, B2).

The subgroup test specimens of (A1, B1and A2, B2) were prepared with standardized disc shape of dimensions (25mm diameter and 3 mm thickness) to evaluate chemical composition of denture base material by using Fourier Transform Infrared Spectroscopy (FTIR) and also the cytotoxicity of these denture base materials.

The specimens of subgroup (A1 and A2) were made from heat polymerized Polymethylmethacrylate acrylic resin. A metal mold made of steel was used to pour a wax pattern. Wax patterns were flasked, dewaxed and packed by using heatpolymerized acrylic resin. Heat polymerized acrylic resin was mixed in accordance with the manufacturer's instructions and placed in the prepared mold and packed in the metal flask. Acrylic resin specimens were processed for 9 hours in water bath and kept at a constant temperature of 165°F (73.5°C). Conventional cutters, trimmers were used for finishing and polishing. Subgroup (B1 and B2) test specimens were prepared from Computer-Assisted Designing (CAD) and Computer-Assisted Milling (CAM) polymerized acrylic resin block denture base material. Polymerized block of CAD/CAM acrylic resin denture base material was cut into the standardized dimensions, with a diamond disk (Isomet, Buehler, USA) under water irrigation. The specimens were finished and highly polished using a polishing machine (1000 grt.) under a water cooling system. The polished specimens were stored in distilled water at 370C for 24 hours.

Fourier transform Infrared spectrophotometer (FTIR) (GroupA1, B1):

An infrared spectroscopy (Shimadzu, IRspectrophotometer-8400S, Kyoto, Japan) was used for analyzing the polymerized specimens of group (A1 and B1). The specimens were ground with 10 times of its bulk of pure potassium bromide (KBr). The mixture of KBrpowder was pressed into a disk using special mold and hydraulic press of the apparatus. The disk specimens were placed on magnetic sheet. The beam enters the sample compartment where it was transmitted through the surface of the sample. The beam finely passed to the detector for final measurement. The detector was designed to measure the special interferogram signal that digitized and sent to the computer where the Fourier transformation takes place. The final IR spectra were collected on a Fourier Transform Infrared (FTIR), using a software program IR solution.

Cytotoxicity assay (Group A2, B2):

Cell treatment

Discs samples of subgroup (A2 and B2) acrylic resin was rinsed in complete culture media for 72 hours to get an extract of the above-mentioned resins and then the media was sterile-filtered before use. Human mesenchymal stem cells (hMSCs) were seeded at 3.5x 104in 35 mm petri dishes and incubated before treatment for 24 hrs. Cells were cultured in the media extract of subgroup (A2) and subgroup (B2) acrylic resin for 72 hrs. Cells cultured in complete culture media served as the negative control.

Cell viability and metabolic activity of cells

To determine whether subgroup (A2) or subgroup (B2) acrylic resin affected cell growth in culture, cellular proliferation experiments were performed with WST-1 assay kit (Roche Diagnostics, Mannheim, Germany) according to the hMSCs' manufacturer's instructions. In brief, cells were seeded at a density of 3.5x104 on a 35 mm Petri dish in complete culture medium for 24 hrs. Next day, the cells were treated with the media extract of either heat polymerized or CAD/CAM acrylic resin for 3 days. For measurements, the medium was replaced by fresh medium supplemented with WST-1 reagent added directly into the incubation media (diluted 1:10 with culture media). After 4 hours of incubating the cells at 37°C in 5%, CO2to form purple formazan crystals, 150 µL of the mixture were transferred into a 96-well culture plate in triplicates. The optical absorbance of the supernatants was determined at 450 nm against a reference wavelength of 620 nm usingMultiskan FC microplate plate reader (ThermoScientific, Massachusetts, USA). The resulting dye was directly correlated with the number of metabolically active cells in the culture. Each plate contained blanks, control and experimental subgroups (A2 and B2) with three replicates each. The blank absorbance was subtracted from those of the sample wells. Cytotoxicity of the compounds was expressed as percentage cell viability compared to controls. The absorbance of cells exposed to normal culture media (negative controls) was taken as 100% cell viability.

Live/Dead Staining

Cell viability was assessed by staining the cells with Calcein-AM/EthD-III using Live/Dead fluorescent Cell Staining Kit II (Promokine, PK-CA707-30002) according to the manufacturer's protocol. In brief, cells were cultured in 35 mm petri dishes (3.5x104 cell/well) for 24 hrs to allow attachment. Cells were then treated with media extract of either subgroup (A2) or subgroup (B2) acrylic resin for 3 days. The cells were washed twice with PBS (phosphate buffered saline) and sufficient volume of Calcein-AM/EthD-III staining solution was added to cover the cell monolayer. The cells were incubated for 30-45 minutes at room temperature protected from light. The samples were then observed under the fluorescent microscope (AxioObserver Z1; Zeiss, Oberkochen, Germany). The experiment was repeated three times from two different cell lots.

Statistical Analysis:

All statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 20 (IBMCorp., Armonk, NY). Descriptive statistics as means and standard deviations were used. For normally distributed data, comparison between the means of the survival cell rate percentage of hMSCs after 24hrs of incubation period was subjected to analysis of two way (ANOVA).

RESULTS

Fourier transform Infrared spectrophotometer (FTIR) analysis:

Infrared spectra analysis displayed several peaks of subgroup (A1and B1), the transmission spectra were recorded in the range of500-4000 $cm^{-1}$and serve as a direct means for the identification of the function group on the surface.

Table 1. FTIR characterization of subgroup (A1) and (B1)

Subgroup (A1):PMMA		
Peak (wave Length)	Assignments	Functional group
3454.62	O-H& N-H	Alcohol &phenols or Amine
2972.40	C-H &O-H	Carboxylic acid
1734.06	C=O& C-O	Aldehydes & carboxylic acid
1456.30	CH2	Alkanes
1190.12	C-O	Alcohol
Subgroup (B1): CAD\CAM		
3439.19	O-H	Alcohol & phenols or Amines
2953.12	C-H &O-H	Carboxylic acid
1734.06	C= O &C-O	Aldehydes Carboxylic acid
1626.05	C=C	Alkenes
1462.09	-CH2	Alkanes
1261.49 - 1151.54	C-O C=O	Alcohol Ketones
976.01	C-O	Anhydriedes
839.06	C-H	Aromatic
754.19	C-CL	Alkylhalides

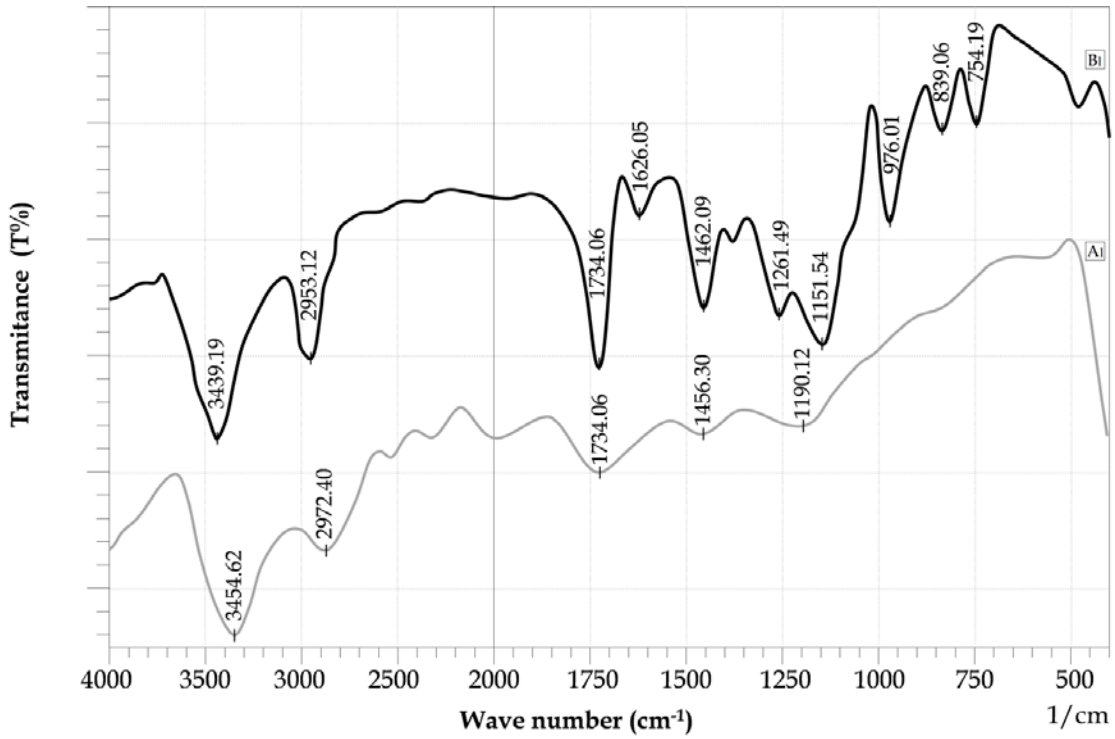


Fig. 1. FTIR of subgroup A1 and B1

Figure (1) and table (1) represented the following:
The transmission peak at 2972.4 cm^{-1} is characteristic for C-H (methylene) & O-H. It is sharp and strong peak in subgroup (B1), while in subgroup (A1) the same peak 2953.12 cm^{-1} displayed a medium intensity peak.

The transmission peak at1734.06 cm^{-1} is characteristic for C=O (ester carbonyl) stretching vibration, strong broad band in subgroup (B1) and changed to medium peak in subgroup (A1).
The peak of 1626.05 cm^{-1} for C=C (carbon-to-carbon double bond) appears only in-group (B) with medium intensity.
The peak at 1462.09 cm^{-1} is characteristic for CH_2 (aromatic group). It is strong broad peak in subgroup (B1) and changed to weak peak in subgroup (A1) at transmission peak 1465.30 cm^{-1}.
The transmission peak at 1261.49- and 1151.54 cm^{-1} is characteristic for (C-O ester band and C=O) in subgroup (B1) changed from double moment peak to weak peak at 1190.21 cm^{-1} in subgroup (A1).
In subgroup (B1) three peaks appeared at 976.01, 839.06 and 754.19 cm^{-1} i.e. a three-added functional group was included in the components of subgroup (B1).
When comparing the functional groups in sub group (B1) with those of subgroup (A1), there was a significant change. It presented an internal difference in the structure of the two subgroups (A1 and B1).

Cytotoxicity assay results (Subgroup A2 and B2):

Cell viability and metabolic activity of cells:

The survival cell rate percentage of hMSCs of subgroup (A2, B2) and the -ve control group after 24 hrs of incubation period is presented in (Fig.2). Comparison between the control group and the tested subgroups (A2 and B2) using two-way ANOVA displayed no statistically significant difference.

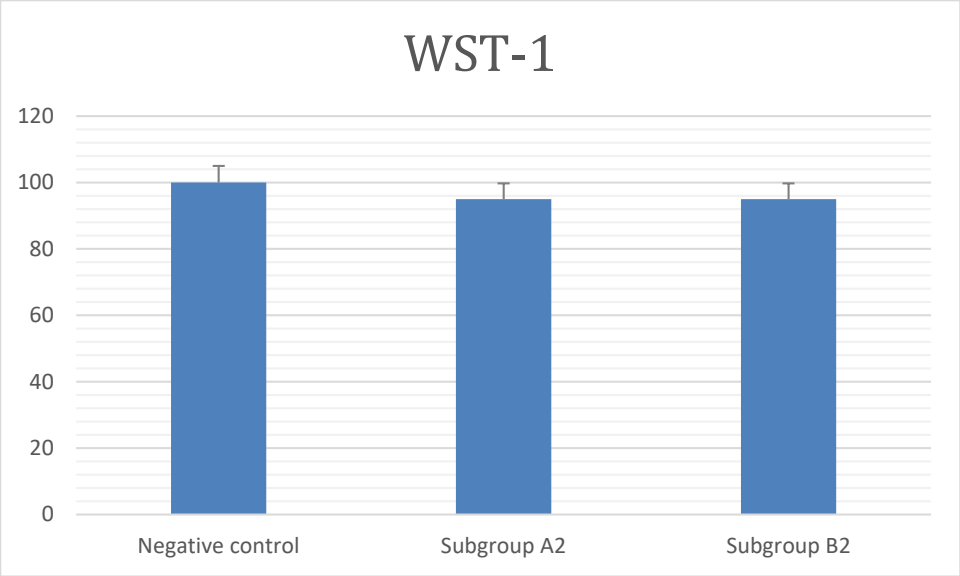
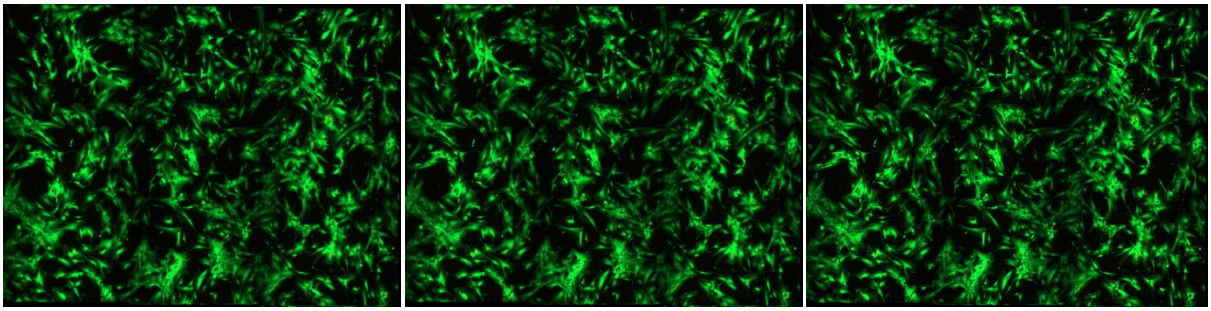


Fig. 2. The survival cell rate percentage of hMSCs of the control group and test subgroups (A2 & B2) after 24 hours of incubation period.

Live/Dead Staining

Viability of human mesenchymal stem cells using the live/dead fluorescence assay were analyzed qualitatively based on the morphological features evident from the photomicrographs. This test displayed that cell viability was not affected by the treatment of the cells with media extract from either subgroup (A2) or subgroup (B2) acrylic resins when compared to the control group. These results were in accordance to the WST-1 results. Live cells, observed in the control group as well as in all treatment subgroups, retained the polyanionic dye calcein and presented an intense green fluorescence (Fig.3) with no morphological changes. There was no evidence of increasing cell death, i.e., the number of cells emitting red fluorescence which would have evidenced penetration of the cell membrane by EthD-1 was not altered.



Control Group Subgroup A2 Subgroup B2

Fig. 3. Live/Dead fluorescent cell viability at 10× magnification

DISCUSSION

CAD/CAM removable denture prosthodontics now automatizes many of the denture construction phases and is believed to produce a more persistent denture excellence. Many properties of dental materials are reliant on the surface structure and on the chemical composition of outer surface layer [16].

FTIR was selected in this study for chemical analysis of the samples due to its powerful analytic technique and its quantitative measure for identification of the composition of the polymer and polymerization of the dental materials. Variation in the molecular components of the materials were reflected as a shift in the absorbance band intensity and its position in the vibration spectra. It presents a sensitive analytic tool to detect composition changed in biomaterials. IR spectrum similarly represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibration between the bonds of the atoms making up the material [17, 18, 19, 20].

The spectra of heat-polymerized resin (subgroup A1) was used as a reference, because it has more favorable chemical properties and to compare with pre-polymerized CAD/CAM acrylic resin (subgroup B1) [16]. The present study of IR spectra of CAD/CAM (group B) presented a presence of small stretch, medium intensity at1626.5cm^{-1} which indicated unpolymerized C=C. The result may be contributed to the reaction of polymerization never reaching 100% conversion i.e. conversion of monomer into polymer is not completed. The degree of conversion is expressed as % of unreacted (C=C) bonds, but the final degree of conversion of resin depends on the chemical structure of monomer i.e. atmosphere, temperature, light intensity and photo initiator concentration [21, 22, 23].

Long polymerization of heat polymerized acrylic denture base (subgroup A1) was used in this study because a lot of residual monomer was lost to the water-immersed mould during the polymerisation process, while in subgroup (B1) the CAD/CAM denture bases are milled from industrially PMMA pucks that have been polymerized under high temperature and pressure resulting in highly condensed resins.

The high pressure promotes the formation of long polymer chains and therefore leads to higher degree of monomer conversion with lower values of residual monomer because the desired zero residual monomer cannot be achieved. These results were in accordance with the study done by Steinmass et al. (2016) who stated that CAD/CAM dentures release very little monomer [24, 16].

Dental literature reveals lack of information regarding the cytotoxicity of CAD/CAM acrylic resin material on the oral tissues. Therefore, in vitro cytotoxicity test was selected as a simple mean of evaluation in this study in an attempt to reduce the effect of cofounding variables. Moreover, to simulate and detect the biological reactions of these materials on human tissues and considered as source of evidence in evaluating cytotoxicity of CAD/CAM denture base resins in removable prosthodontics before its clinical application [24].

Cytotoxicity of subgroup (A2and B2) denture base resin was assessed using WST-1 assay and fluorescent live/dead assay for the measurement of cell proliferation and cell viability. The extent of influence and sensitivity of cytotoxicity depends on the denture base resin and the cell culture [25, 26, 27, 28, 10]. Cytotoxic WST-1 assay test was carried out in the present study after 24 hrs. of incubation period because reports of cytotoxic effect of acrylic resin revealed that a release of residual monomer is more intense in the first 24 hrs. and is responsible for reduction of cell viability [24, 29, 30, 31].

Human Mesenchymal stem cells (hMSCs) were selected as the cell culture. They are used in the protocol line of cytotoxicity test because the denture base resin materials are in contact with the tissue. hMSCs are multipotent self-renewing progenitor cells with the ability to secrete growth factors, cells capable of differentiating into several cell

lineages including osteoblasts, chondrocytes, and adipocytes, easy isolation and expansion, unique anti-inflammatory and immune-modulatory properties [32,33,34].

The results of survival cell rate percentage of hMSCs at WST-1 after 24 hrs. incubation period revealed no statistical significant difference between the negative control and the tested subgroups (A2& B2). This result may be due to the residual monomer starts to leak in water and resulting of less monomer that cause cell damage and several methods may be taken to reduce the amount of monomer, such as polymerization in water or under pressure, or the use of correct polymer, monomer proportion and storage in water for 72 hrs. after polymerization [24,31]. The small stretch of unpolymerized (C=C) of CAD/CAM of subgroup (B1) had no significant effect on cells on both WST-1 and the live/dead fluorescence assay.

Live/dead fluorescence assay is a fluorescent marker which are used to support the finding of WST-1 assay. Live /dead fluorescence assay provides an accurate index of cell viability and proliferation and considered to be more sensitive than WST-1assay for evaluating the cytotoxicity of these denture base resin. Fluorescence microscope was used with Live /dead fluorescence assay to provide greater resolution and imaging of biological structures than conventional imaging [35]. The Live /dead fluorescence assay results were in accordance with the results of WST-1.

CONCLUSIONS

According to the methodology used in this study, FTIR spectroscopy analysis indicated the presence of very small amount of residual monomer in CAD/CAM denture base material that was nearly at the same level of heat polymerized type with no significant difference. Moreover, to detect the cytotoxic effect of residual monomer, two sensitive methods of cell viability were used to distinguish the biocompatibility of the two different types of polymerized acrylic denture bases. Both tests revealed that the tested groups displayed no cytotoxic effect on the viability of hMSCs after 24 hrs. incubation period.

REFERENCES

1. Cheng Y.Y., Cheung W.L., Chow T.W. Strain analysis of maxillary complete denture with three-dimensional finite element method. J. Prosthet. Dent. 2010; 103:309–318.
2. Hirajima Y., Takahashi H., Minakuchi S. Influence of a denture strengthener on the deformation of a maxillary complete denture. Dent. Mater. 2009; 28:507–512.
3. Seo R.S., Murata H., Hong G., Vergani C.E., Hamada T. Influence of thermal and mechanical stresses on the strength of intact and relined denture bases. J. Prosthet. Dent. 2006; 96:59–67.
4. Ajaj-ALKordy N.M, AlsaadH.M: Elastic modulus and flexural strength comparisons of high-impact and traditional denture base acrylic resins The Saudi Dental Journal 2014 ,26: 15–18.
5. Johnston E.P., Nicholls J.I., Smith D.E. Flexure fatigue of 10 commonly used denture base resins. J. Prosthet. Dent. 1981; 46:478–483.
6. Kondo Y, Tomohiro Takagaki T., Okuda M., Ikeda M., Kadoma Y., Yamauchi J., Okada K., Sadr A., Nikaido T. and TagamiJ., Effect of PMMA filler particles addition on the physical properties of resin Composite. Dental Materials Journal 2010; 29(5): 596–601.
7. kattadiyilM., Goodacre Ch. J. and Baba N.Z.: CAD/CAM Complete Dentures: A Review of Two Commercial Fabrication Systems. California Dental Association (CDA) Journal 2013: l 4 1; 6.
8. Baba N.Z: Materials and Processes for CAD/CAM Complete Denture Fabrication. Curr Oral Health Rep. 2016:3:203–208.
9. Amin M., Akbar M. and Amin S.: Hydrophobicity of silicone rubber used for outdoor insulation (An overview) Rev. Adv. Mater. Sci., 2007:16; 10-26.
10. Huang FM, Hu CC, Chang YC, Chou MY. The level of monomer release from acrylic denture base materials into water. Chin Dent J. 2000; 19:17–22.
11. Vallittu PK. The effect of surface treatment of denture acrylic resin on the residual monomer content and its release into water. Acta Odontol Scand. 1996 Jun;54(3):188–92
12. Ferracane, J.L. Resin composite—State of the art. Dent. Mater. 2011, 27, 29–38.
13. Stawarczyk B., Teuss S., Eichberger M., Roos M. and Keu Ch. Retention Strength of PMMA/UDMA-Based Crowns Bonded to Dentin: Impact of Different Coupling Agents for Pretreatment. Materials 2015, 8, 7486–7497.

14. Stawarczyk B., Sener B., Trotimanni A., Roos M., Özcani M., and Hammerle Ch.H. F: Discoloration of manually fabricated resins and industrially fabricated CAD/ CAM blocks versus glass-ceramic: Effect of storage media, duration, and subsequent polishing. Dental Materials Journal 2012; 31.
15. Stawarczyk B, Ender A, Trottmann A, Özcan M, Fischer J, Hämmerle CH. Load-bearing capacity of CAD/CAM milled polymeric three-unit fixed dental prostheses: effect of aging regimens. Clin Oral Invest. 2012; 16:1669-77.
16. Steinmass P.A., Wiedemair V., Huck Ch., Klaunzer F., Steinmass O., Grunert I., DumfahrtH.: Do CAD/CAM dentures really release less monomer than conventional dentures? Clin Oral Invest 2016: 1961-6.
17. Stuart B. Infrared Spectroscopy: Fundamentals and Applications.2004; Pp: 113-135.
18. Bhargava R, Wang S, and Koenig J. FTIR Microspectroscopy of Polymeric Systems. AdvPolym Sci. 2003; 163:137–191.
19. Barbara S. Infrared Spectroscopy, Kirk-Othmer Encyclopedia of Chemical Technology 2005 :15: 4.
20. Huang FM., Hu CC., and Chang YC.: Residual monomer releasing from acrylic denture base in water. Chin Dent. J.2000; 19:17-22.
21. Al-Ali AAS, Kassab-Bashi TY: Fourier Transform Infra-Red (FTIR) Spectroscopy of new copolymers of acrylic resin denture base materials. International Journal of Enhanced Research in Science Technology & Engineering,2015 ,4:4, pp: (172-180).
22. Urban V, Mashado A, Vergani C, Jorgeem G, Santosm L, Leite E, and Canevarolo S.: Degree of conversion and molecular weight of denture base and three reline submitted to post- polymerization treatment. Mat Res. 2007; 10:1-13.
23. Zissis A, YannikakisS, Polyzois G, Harrison A: A long term study on residual monomer release from denture materials. Eur J Prosthodont Restor Dent 2008, 16:81–84.
24. Saravi M.E , Vojdani M , and Bahrani F.: Evaluation of Cellular Toxicity of three denture base acrylic resins. J Dent (Tehran). 2012; 9(4): 180–188.
25. Bal B.T., Yılmaz H., Aydın C., Karakoca S., Yılmaz S.: In Vitro cytotoxicity of maxillofacial silicone elastomers: effect of Accelerated Aging J Biomed Mater Res Part B: Appl Biomater 89B: 2009 ;122–126.
26. Banada PP, Panbangred W, Bhunia AK.: WST-1based cell cytotoxicity assay as a substitute for MTT basedassay for rapid detection of toxigenic bacillusspecies. Journal of Microbiological Methods 2008, 73(3):211-215.
27. Tayel BS and Alammari M R: Cellular Response to Pigmented Silicon Maxillofacial Prosthetic Material. Life Science Journal 2014; 11(9).
28. Niu Q, Zhao C, Jing Z: An evaluation of the colorimetric assays based on enzymatic reactions used in the measurement of human natural cytotoxicity. J Immunol Methods 2001, 251(1): 11-19.
29. Ozdemir KG, Yilmaz H, Yilmaz S: In vitro evaluation of cytotoxicity of soft lining materials on L929 cells by MTT assay. J Biomed Mater Res B Appl Biomater. 2009, 90(1):82-6.
30. 1Prosthodontist, Department of Prosthodontics, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran Gonçalves TS, Menezes LM, Silva LE. Residual monomer of autopolymerized acrylic resin according to different manipulation and polishing methods. Angle Orthod. 2008; 78(4):722-7.
31. Retamoso L. B, da Cunha M.A., Pithon M.M, dos Santos R.L., Martins F.O., Romanos M.VT. V, Tanaka M.O. In vitrocytotoxicity of self-curing acrylic resins of different colors J Orthod. 2014, 19(4):66-70.
32. Ryan JM, Barry FP, Murphy JM, Mahon BP: Mesenchymal stem cells avoid allogeneic rejection.Journal of Inflammation 2005, 2:8.
33. Wong V, Levi B, Rajadas J., Longaker M., and GurtnerG.: Stem Cell Niches for Skin Regeneration.International Journal of Biomaterials. 2012, 1-8.
34. MaxsnC, Lopez E.A., Yoo D., Miagkova A.D., LerouxM. A.: Concise review: Role of mesenchymal stem cells in wound repair. Stem cells translational medicine 2012; 1:142-149.
35. D'Alpino, P.H.P., Pereira, J.C., Svizero, N.R., Rueggeberg, F.A. & Pashley, and D.H: Use of fluorescent compounds in assessing bonded resin-based restorations: A literature review. J Dent .2006:34; 623–634.