Cytotoxicity of Soft Denture Lining Materials Depending on Their Component Types

Yo-Han Song, MSc[a]/Ho-Jun Song, PhD[b]/Mi-Kyung Han, PhD[c]/
Hong-So Yang, DDS, PhD[d]/Yeong-Joon Park, DDS, PhD[e]

Purpose: To evaluate the difference in cytotoxicity of soft denture lining materials depending on their component types. ***Materials and Methods:*** Ten commercially available soft denture lining materials (SDLM) consisting of five silicone-based materials and five acrylic-based materials were evaluated. For the MTT test, cured SDLM samples were extracted in a culture medium for 24 hours, and L-929 cells were incubated in the extracted medium for 24 hours. Cell viability was determined using a microplate reader and compared with those of the negative control, which were cultured in a culture medium without test material. Agar overlay test was performed for the cured SDLM samples according to International Organization for Standardization (ISO) 7405. ***Results:*** Among silicone-based lining materials, GC Reline Soft, Mollosil plus, and Dentusil showed a cell viability of 107.2% ± 4.5%, 102.3% ± 2.84%, and 93.0% ± 8.0%, respectively, compared with the control. Mucopren and Sofreliner Tough displayed significantly lower cell viability (86.4% ± 10.3% and 81.5% ± 4.3%, respectively) compared with the control ($P < .05$). Among acrylic-based materials, Kooliner, Visco-gel, Soft liner, Dura Base, and Coe-Soft displayed cell viability of 99.2% ± 14.6%, 93.1% ± 9.5%, 89.1% ± 9.8%, 87.6% ± 7.9%, and 75.9% ± 15.7%, respectively, compared with the control. Dura Base and Coe-Soft displayed significantly lower cell viability compared to the control. However, for all tested materials, cell viability exceeded the requirement limit of 70% specified in ISO 10993-5. In the agar overlay test, all five silicone-based materials and acrylic-based Kooliner were ranked as "noncytotoxic." However, Visco-gel was ranked as "mildly cytotoxic," and Soft liner, Coe-Soft, and Dura Base were ranked as "moderately cytotoxic." ***Conclusion:*** When an acrylic-based soft denture lining material is used, the possibility of a cytotoxic effect should be considered. *Int J Prosthodont 2014;27:229–235. doi: 10.11607/ijp.3848*

If the fit of a denture is reduced due to resorption of the residual ridge and inflammation of supporting soft tissue, the adaptation of the denture to the supporting oral structure and mastication function are reduced. The denture then needs to be lined with soft or hard lining materials to improve its capability.[1–3] Lining materials are classified as short-term use or long-term use, depending on the expected period of adequate functional contact with the denture-supporting mucosa. Long-term use materials are used for more than 28 days, and short-term use materials are used for a limited period of up to 7 days to improve fit, retention, and comfort.[4,5]

The commonly used soft liners are either plasticized acrylic resins or polydimethylsiloxane, to which filler is added to provide the correct consistency.[6] The silicone-based lining materials do not have plasticizers and retain their resilient properties for a longer period.[6] Since the denture liners are in direct contact with oral mucosa, they should be nonirritating, nontoxic, and resistant to bacterial and fungal colonization.[1]

There have been several reports describing chemical irritation or tissue hypersensitivity of denture-bearing soft tissues after insertion of dentures,[7–12] and estrogenic compounds, such as phthalate plasticizers

[a]*Graduate Student, Department of Dental Materials and Research Center for Biomineralization Disorders, School of Dentistry, Chonnam National University, Gwangju, Korea.*
[b]*Associate Professor, Department of Dental Materials and Research Center for Biomineralization Disorders, School of Dentistry, Chonnam National University, Gwangju, Korea.*
[c]*Research Professor, Department of Dental Materials and Research Center for Biomineralization Disorders, School of Dentistry, Chonnam National University, Gwangju, Korea.*
[d]*Professor, Department of Prosthodontics, School of Dentistry, Chonnam National University, Gwangju, Korea.*
[e]*Professor, Department of Dental Materials and Research Center for Biomineralization Disorders, School of Dentistry, Chonnam National University, Gwangju, Korea.*

Correspondence to: *Dr Yeong-Joon Park, Department of Dental Materials, School of Dentistry, Chonnam National University, 300 Yongbong-dong, Buk-gu, Gwangju 500-757, South Korea. Fax: +82-62-530-4875. Email: yjpark@jnu.ac.kr*

Table 1 Tested Materials

Component	Product	Manufacturer	Lot no.	Composition
Silicone-based				
	Dentusil	Bosworth (USA)	1112-583	Self-cure vinyl polysiloxane
	GC Reline Soft	GC Dental Products (Japan)	1109061	Self-cure vinyl polysiloxane
	Mollosil plus	Detax GmbH & Co (Germany)	140202	Self-cure vinyl polysiloxane
	Mucopren	Kettenbach GmbH & Co (Germany)	100232	Self-cure vinyl polysiloxane
	Sofreliner Tough	Tokuyama Dental Corporation (Japan)	081	Self-cure vinyl polysiloxane
Acrylic-based				
	Coe-Soft*	GC America (USA)	1105271	Powder: acrylic polymer, ZU (1% to 5%) Liquid: acrylic monomer, BS (35% to 40%), EtOH (10% to 15%)
	Dura Base	Reliance Dental Mfg (USA)	Powder: 021210 Liquid: 031010	Powder: PMMA particulates Residual monomers (< 1%) Dialkylphthalate (< 15%) Trade secret (< 5%) Liquid: methyl methacrylate monomer (100%)
	Kooliner*	GC America (USA)	1110181	Powder: PEMA (88%), BP (1% to 5%) Silica (9% to 12%) Liquid: IM (60% to 100%), DB (1% to 5%)
	Soft liner*	GC Dental Products (Japan)	Powder: 1202081 Liquid: 1202061	Powder: acrylic polymer Liquid: acrylic monomer, EtOH (15%)
	Visco-gel*	Dentsply (USA)	1203000293	Powder: PEMA (50% to 100%) Liquid: EtOH (2.5% to 10%), citrate ester plasticizer, mineral oil, spearmint oil

*Materials used for short term. Other materials are used for long term; ZU = zinc undecylenate; BS = benzyl salicylate; EtOH = ethyl alcohol; PMMA = poly(methyl methacrylate); PEMA = poly(ethyl methacrylate); BP = benzoyl peroxide; IM = isobutyl methacrylate; DB = 2,4-dihydroxy benophenone.

leaching from dental polymers, have evoked concern regarding biologic safety.[13–16] An in vitro cytotoxicity study involving nine different soft and hard lining materials reported potent cytotoxicity for a product showing a cell viability of less than 10%.[17] Another study that examined five different soft lining materials also reported strong cytotoxicity for one product, with less than 40% of cell viability.[18] These results have prompted concern over the use of denture lining materials that are used in direct contact with large areas of oral mucosa.

There are additional new commercially available lining materials that need to be scrutinized for their biologic safety. In this study, 10 commercially available soft denture lining materials (5 silicone-based and 5 acrylic-based) were evaluated by MTT and agar overlay testing.

Materials and Methods

Component types, product names, manufacturers, lot numbers, compositions, and usage duration types for the tested materials are listed in Table 1.

Specimen Preparation

Preparation of the test specimens of soft liner materials and measurement were performed according to International Organization of Standardization (ISO) 10993-5.[19] Five types of silicone-based soft lining materials (Dentusil, GC Reline Soft, Mollosil plus, Mucopren, and Sofreliner Tough) and five types of acrylic-based soft lining materials (Coe-Soft, Dura Base, Kooliner, Soft liner, and Visco-gel) were examined. Eight specimens per each material type were prepared in Teflon molds of 5-mm diameter and 2-mm thickness. After overfilling the mold cavity, excess material was removed with pressure exerted on a cover glass, and the test samples were cured according to the manufacturers' instructions. The specimens were aged for 24 hours in sterilized water and exposed to ultraviolet light for 30 minutes to prevent bacterial contamination.[20]

Preparation of Extracts

Extracts were prepared from cured specimens according to ISO 10993-12,[21] using RPMI1640 medium supplemented with 100 units/mL of penicillin, 100 mg/mL of streptomycin, and 10% fetal bovine serum (FBS) (Gibco BRL) for the MTT test. The extraction medium containing the sample was shaken in an incubator at 37°C. The medium extracts were incubated for 30 minutes at 37°C in 5% CO_2 to stabilize the pH. Medium without a sample disk was also prepared to serve as the negative control.

MTT Test

Cell viability was assessed by the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to insoluble formazan. L-929 mouse fibroblast cells were grown on a 100-mm-diameter Petri dish containing 10 mL RPMI1640 supplemented with 10% FBS. When sufficient cells were produced, 100 µL of the cell suspension (3 × 103 cells/mL) were seeded in wells of a 96-well plate and incubated at 37°C in 5% CO_2. After 24 hours, the culture medium was removed from the wells and an equal volume (100 uL) of the prepared extract was added into each well and cultured for 24 hours. The test extracts were removed, and 100 µL/well medium and 10 µL MTT solution were added to each well and kept in a dark environment for 4 hours at 37°C. The MTT was aspirated, and 100 µL/well of dimethylsulfoxide (Sigma-Aldrich) was added to each well. The plate was shaken for 15 minutes, and absorbance generated by the insoluble formazan was measured at 570 nm by a microplate reader (Bio-Rad Laboratories). Five separate analyses were performed. Cytotoxicity was rated based on cell viability relative to controls as: noncytotoxic (> 90% cell viability), slightly cytotoxic (60% to 90% cell viability), moderately cytotoxic (30% to 59% cell viability), and severely cytotoxic (< 30% cell viability).[22]

Agar Overlay Test

Five milliliters of L-929 cell suspension (3 × 105 cells/mL) were seeded in 50-mm-diameter cell culture dishes and incubated to confluence at 37°C in 5% CO_2. After 24 hours incubation, the medium was replaced with 5 mL of freshly prepared agar/nutrient medium containing RPMI1640, 5% FBS, and 3% agarose mixture. A 5-mL neutral red solution (0.01% in phosphate-buffered saline, Sigma-Aldrich) was added, and the cells were incubated for 15 minutes at room temperature. Excess dye was removed, and the test specimens were placed on the agar surface. A 0.25% zinc dibutyl dithiocarbamate polyurethane film was used as positive control and a polyethylene sheet as negative control. The dishes were incubated for 24 hours at 37°C in 5% CO_2. The cultures were examined by light microscopy. The decolorized zones and cell lysis around and/or under the specimens were evaluated according to ISO 7405.[23] Each test was repeated three times. The decolorized zones were scored as 0 (no decolorization detectable), 1 (decolorization only under the specimen), 2 (decolorization zone not greater than 5 mm from the specimen), 3 (decolorization zone not greater than 10 mm from the specimen), 4 (decolorization zone greater than 10 mm from the specimen), and 5 (total culture decolorized). Cell lysis was defined as loss of cell membrane integrity. Cell lysis was scored as 0 (no cell lysis detectable), 1 (< 20% cell lysis), 2 (20% to 40% cell lysis), 3 (> 40% to < 60% cell lysis), 4 (60% to 80% cell lysis), and 5 (> 80% cell lysis). For each specimen, the median score from each specimen was calculated for both the decolorization zone index and the lysis index. Cell response was classified as follows: noncytotoxic 0 to 0.5, mildly cytotoxic 0.6 to 1.9, moderately cytotoxic 2.0 to 3.9, and markedly cytotoxic 4.0 to 5.0. The median values were calculated to describe the central tendency of the scores because the results were expressed as an index in a ranking scale.[22,23]

Statistical Analyses

SPSS version 20.0 (IBM) was used to assess the data of the MTT test with Kruskal–Wallis one-way analysis of variance and Duncan's multiple range test. The results were expressed as the mean ± SD for five separate experiments. A P value < .05 was considered statistically significant.

Results

The results of the MTT test using extracts of the test materials are given in Table 2 and Fig 1. Among the examined silicone-based lining materials, GC Reline Soft, Mollosil plus, and Dentusil were noncytotoxic, with cell viability of 107.2% ± 4.5%, 102.3% ± 2.84%, and 93.0% ± 8.0%, respectively, compared with the control.[22] Mucopren and Sofreliner Tough were ranked as slightly cytotoxic, showing a significantly lower cell viability (86.4% ± 10.3% and 81.5% ± 4.3%, respectively, compared with the control; P < .05).

Among the acrylic-based materials, Kooliner and Visco-gel were noncytotoxic, showing a cell viability of 99.2% ± 14.6% and 93.1% ± 9.5%, respectively. Soft liner, Dura Base, and Coe-Soft were ranked as slightly cytotoxic, showing a cell viability of 89.1% ± 9.8%, 87.6% ± 7.9%, and 75.9% ± 15.7%, respectively. The cell viabilities of Dura Base and Coe-Soft were significantly lower than that of the control group (P < .05).

However, all the tested materials showed a cell viability higher than the requirement limit of ISO standard.[19]

In the agar overlay test, all five silicone-based materials and acrylic-based Kooliner were ranked as noncytotoxic. However, among the acrylic-based materials, Visco-gel was ranked as mildly cytotoxic, and Soft liner, Coe-Soft, and Dura Base were ranked as moderately cytotoxic (Table 3, Fig 2).

Table 2 Percent Cell Viability for Soft Lining Materials After Cell Culture for 24 h*

Component	Material	Mean ± SD
	Control	100 ± 0.0[a,b,c]
Silicone-based		
	Dentusil	93.0 ± 8.0[b,c,d]
	GC Reline Soft	107.2 ± 4.5[a]
	Mollosil plus	102.3 ± 2.8[a,b]
	Mucopren	86.4 ± 10.3[d,e]
	Sofreliner Tough	81.5 ± 4.3[d,e]
Acrylic-based		
	Coe-Soft	75.9 ± 15.7[e]
	Dura Base	87.6 ± 7.9[d,e]
	Kooliner	99.2 ± 14.6[a,b,c]
	Soft liner	89.1 ± 9.8[c,d,e]
	Visco-gel	93.1 ± 9.5[b,c,d]

*N = 5.
By Kruskal-Wallis statistics: K= 30.192, $P = .000$, $P < .05$.
Means with the same letter are not significantly different ($P > .05$).
Duncan post hoc grouping: a > b > c > d > e.

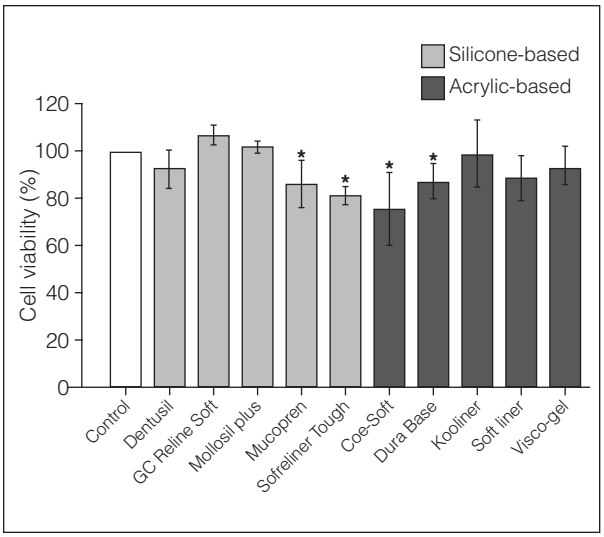

Fig 1 Mean ± SD cell viability on soft denture lining materials vs control after cell culture for 24 hours (n = 5). *Significantly different from the control, $P < .05$.

Table 3 Cytotoxicity of Soft Lining Materials Evaluated by the Agar Overlay Test*

Component	Material	DI	LI	Cytotoxicity
Polyurethane	Positive control	2	2	Moderate
Polyethylene	Negative control	0	0	None
Silicone-based				
	Dentusil	0	0	None
	GC Reline Soft	0	0	None
	Mollosil plus	0	0	None
	Mucopren	0	0	None
	Sofreliner Tough	0	0	None
Acrylic-based				
	Coe-Soft	3	2	Moderate
	Dura Base	3	3	Moderate
	Kooliner	0	0	None
	Soft liner	2	2	Moderate
	Visco-gel	1	1	Mild

DI = Decolorization Index; LI = Lysis Index.
*N = 3.

Discussion

Since the absorption of certain substances released from material in a patient's body can be toxic at high concentrations, verifying the biologic and toxicologic safety of dental materials is a prerequisite for clinical use. In vitro cytotoxicity tests are simple, reproducible, cost effective, and suitable for use in evaluating the basic biologic properties of dental materials.[1,24] In vitro assays for initial screening of dental materials have the advantage of easy control of experimental factors that are often a problem when performing experiments in vivo.[1,24]

In this study, the cytotoxicity of 10 soft lining materials was evaluated using the MTT test and agar overlay test. In the MTT viability test, the activity of the cellular dehydrogenase was measured. The cellular dehydrogenase transforms the tetrazolium salt into an insoluble formazan compound. If the cells are damaged by a toxic substance, an alteration in the permeability of the lysosomal and mitochondrial membranes occurs. As a result, the amounts of enzymes present in these organelles change. Using a suitable marker enzyme, such as cellular dehydrogenase, cell viabilities can be assessed.[25] If the material releases a toxic substance, the cellular dehydrogenase of the contacting cells becomes inactive and formazan does not form. By measuring the amount of formazan, the metabolic activity of the cells can be measured.[26] The agar overlay test measures the cytotoxicity of diffused substances released from a material. Neutral red is a weak cationic dye that readily diffuses across the plasma and organelle membranes, accumulating in the lysosomes. Any loss of membrane integrity induced by a toxic substance will result in decreased retention of neutral red dye. Damaged or dead cells are decolorized in comparison to healthy control cells.[27]

In the MTT test results for acrylic-base lining materials, the cell viability for Coe-Soft and Dura Base was 75.9% ± 15.7% and 87.6% ± 7.9%, respectively, which were significantly lower compared with the control group ($P < .05$; Table 2, Fig 1). In the results of MTT assay by Ozdermir et al[18] for five soft lining materials, Coe-Soft showed cell viability lower than 50% in 24-, 48-, 72-, and 96-hour periods' extracts, which demonstrates a significantly high cytotoxicity

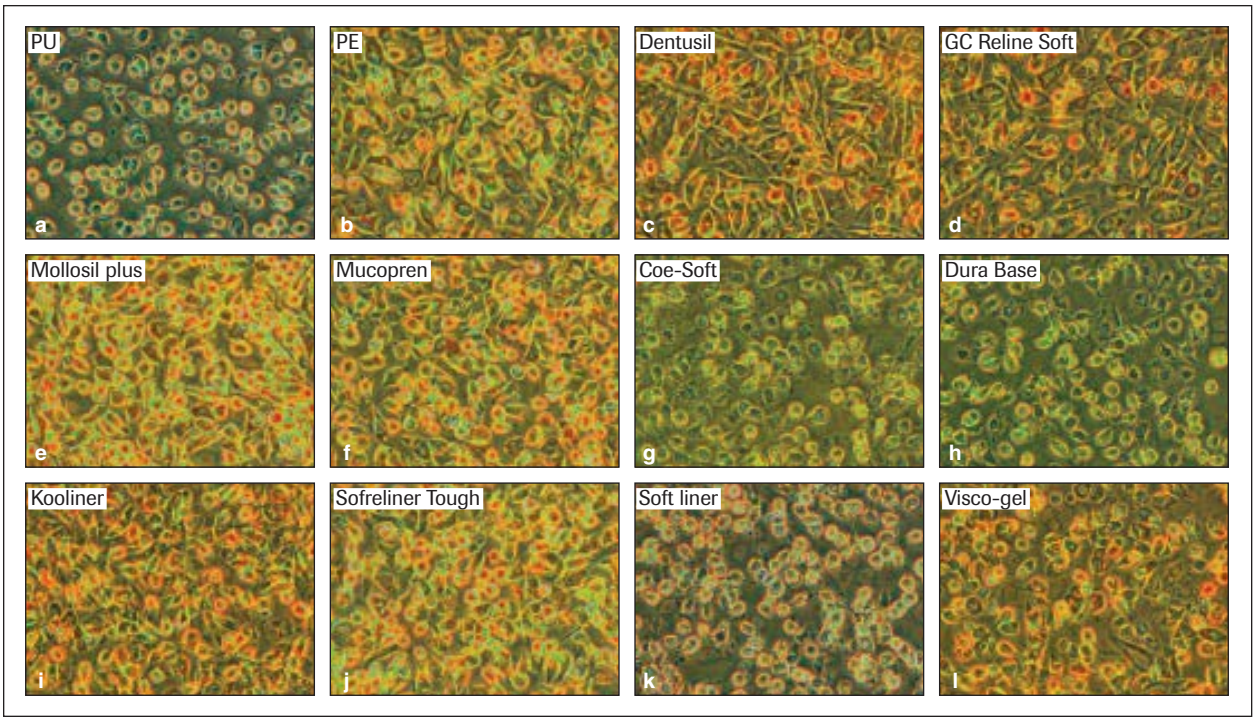

Fig 2 L929 cells in the decolorized zone around the test materials in agar overlay test (original magnification ×200). **(a)** PU = polyurethane (positive control), **(b)** PE = polyethylene (negative control), and **(c to l)** = tested soft denture lining materials.

compared with those of other tested materials. The authors assumed that the cytotoxic effect of Coe-Soft, an acrylic-based soft lining material, was due to the leached aromatic ester component existing in the material. Munksgaard[28] reported that the release of dibutylphthalate plasticizer from Coe-Soft within the first day exceeded the proposed tolerable daily intake for an average adult by 11-fold. Phthalates and other esters of aromatic carboxylic acids are used as plasticizers in acrylic soft lining materials. Some people can become sensitized to benzyl salicylate (BS).[29] BS is contained in Coe-Soft liquid at a concentration of 35% to 40%. The high cytotoxicity of Coe-Soft presently observed may have reflected BS leaching. In this agar overlay test result, the decolorization index for Coe-Soft was ranked as 3, indicative of the more extensive diffusion of the toxic substance than other materials (Table 3). The lysis index for Coe-Soft was 2 and the cells in the decolorized zone changed to a round shape, demonstrating cell death (Fig 2g). Dura Base, which contains dialkylphthalate at a concentration < 15%, displayed moderate cytotoxicity in the agar overlay test (Tables 1 and 3). It was presumed that the leached components from Coe-Soft and Dura Base adversely affected the cytotoxicity of those materials. Interestingly, Kooliner ranked as noncytotoxic in the agar overlay test and produced the highest cell viability (99.2% ± 14.6%) among the acrylic-based materials. Phthalate plasticizer is not included in the Kooliner, and isobutyl methacrylate is included in the liquid instead. Isobutyl methacrylate can be considered as an internal plasticizer responsible for the softness of the material. Since isobutyl methacrylate would be included in the polymer network after curing, leaching of it is not feasible and, as a result, low cytotoxicity of Kooliner could be expected. However, even though short-term use of Kooliner showed no evidence of cytotoxicity in this study, which is exceptional among acrylic-based soft liners (Tables 2 and 3), these results need to be interpreted cautiously regarding long-term use, in light of the report of a strong allergic reaction of Kooliner in the patch test.[30]

Among the tested acrylic-based liners, Coe-Soft, Soft liner, and Visco-gel, which all contain ethanol, showed a decolorization index of 3, 2, and 1, respectively. Ethanol, which is included in the soft liners to produce a short gelation time and large flow after gelation, leaches into saliva, as does the ester plasticizers.[31] The authors assumed that leached residual monomers and plasticizers, and also ethanol, could contribute to the cytotoxicity of the acrylic-based soft lining materials.

In the MTT test conducted with the silicone-based lining materials, GC Reline Soft, Mollosil plus, and Dentusil were ranked as noncytotoxic, showing a cell viability of 107.2% ± 4.5%, 102.3% ± 2.84%, and 93.0% ± 8.0%, respectively, compared with the control. Mucopren and Sofreliner Tough were ranked as slightly cytotoxic, showing a significantly lower cell viability (86.4% ± 10.3% and 81.5% ± 4.3%, respectively) compared with the control ($P < .05$; see Table 2, Fig 1). In the agar overlay test, however, all five of the tested silicone-based materials were ranked as noncytotoxic (see Table 3). In the study of Ciapetti et al,[32] various vinylpolysiloxane impression materials, which are similar to the soft liners in composition, were nontoxic even after prolonged exposure to the cells.

Residual monomer is the component most often cited as an irritant in denture base resins. The residual monomer content of a properly processed denture is < 1%. Furthermore, surface monomer is eliminated following storage in water.[1] In clinical use, the fabricated denture is stored in water for 24 hours before placement in the patient's mouth to reduce biologic complications by leaching out the residual monomer before delivery. Moreover, the volume of the cytotoxic components leached from material will significantly diminish before they pass through the oral mucosa due to dilution by saliva. However, with the limitation of this in vitro study, it is advisable to exercise caution about the cytotoxicity of acrylic-based soft liners. Verifying the safety of Dura Base, a long-term use acrylic-based soft liner, in clinical use needs to be scrutinized.

Conclusion

Even though the cell viability of all tested materials was higher than the minimum requirement limit of ISO standard, some of the materials showed significantly lower cell viability compared with the control group. Especially in the agar overlay test, acrylic-based soft denture lining materials showed mild to moderate cytotoxicity, except for Kooliner. The results suggest that caution is advisable considering the possible cytotoxic effect of soft denture lining materials.

Acknowledgments

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Literature Abstract

Oral bisphosphonate–related osteonecrosis of the jaws in dental implant patients: A case series

The authors reviewed retrospectively the cases of dental implant patients who had taken oral bisphosphonates and subsequently developed bisphosphonate-related osteonecrosis of the jaws (BRONJ) within the 3-year period immediately preceding the writing of the paper. A review of the patient records from 3 hospitals in Galicia, Spain, yielded 9 white patients (8 women and 1 man, with a mean age of 66 years) fitting the description, who altogether had 57 (28 maxillary and 29 mandibular) dental implants placed. The most common reason for taking bisphosphonates was osteoporosis (n = 7). The mean interval between the commencement of bisphosphonate treatment and the onset of BRONJ was 60 months. The average time between dental implant placement and onset of BRONJ was 34 (range, 1 to 96) months. Most lesions were located around mandibular implants (n = 8). Of the 9 patients, 7 recovered completely after treatment. Authors commented that the limited number of subjects did not allow them to assess the contribution of coexisting conditions such as systemic hypertension, corticosteroid medication, or smoking as predisposing factors. The authors admitted that data on the prevalence of BRONJ were not available but thought that the prevalence could be higher than expected. They added that the clinical characteristics of BRONJ lesions and their treatment outcomes were similar to those observed in patients without dental implants.

López-Cedrún JL, Sanromán JF, García A, Penarrocha M, Feijoo JF, Limeres J, Diz P. *Br J Oral Maxillofac Surg (*2013), http://dx.doi.org/10.1016/j.bjoms.2013.06.011. **References:** 24. **Reprints:** Dr JL López-Cedrúna, Stomatology Department, School of Medicine and Dentistry, University of Santiago de Compostela, c/ Entrerríos sn, 15782 Santiago de Compostela, Spain. E-mail: lopezcedrun@centromaxilofacial.com
—*John Chai, Evanston, Illinois, USA*.