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ORIGINAL ARTICLE

Cytotoxicity of denture adhesives

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Abstract Ten commercially available denture adhesives, nine soluble formulations (six creams, three powders) and one insoluble product (pad), were analyzed regarding the cytotoxicity profile in direct and indirect assays using L929 fibroblast cells. In the direct assay, fibroblasts were seeded over the surface of a thick adhesive gel (5%, creams; 2.5%, powders and pad). In the indirect assay, cells were cultured in the presence of adhesive extracts prepared in static and dynamic conditions (0.5–2%, creams; 0.25–1%, powders and pad). Cell toxicity was assessed for cell viability/proliferation (MTT assay) and cell morphology (observation of the F-actin cytoskeleton organization by confocal laser scanning microscopy). Direct contact of the L929 fibroblasts with the thick adhesive gels caused no, or only a slight, decrease in cell viability/proliferation. The adhesive extracts (especially those prepared in dynamic conditions) caused significantly higher growth inhibition of fibroblasts and, in addition, caused dose- and time-dependent effects, throughout the 6–72 h exposure time. Also, dose-dependent effects on cell morphology, with evident disruption of the F-actin cytoskeleton organization, were seen in the presence of most adhesives. In conclusion, the adhesives possessed different degrees of cytotoxicity, but similar dose- and time-dependent biological profiles.

Keywords Prosthetics · Dentures · Denture adhesives · Cytotoxicity · Fibroblasts · Direct assay · Indirect assay

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Introduction

Denture adhesives can enhance prosthesis retention, stability, and function. Industry reports estimate that between 15% and 33% of denture wearers use denture adhesives on a regular basis [1, 2]. In the USA alone, the use of complete dentures was projected to increase from 53.8 million in 1991 to 61.0 million in 2020 [3].

Although the use of adhesives continues to grow, dental professionals generally have had somewhat negative attitudes towards them and often suggested that the use of supplementary retentive appliances is a sign of inadequacy or failure of the oral rehabilitation [1, 4]. In addition, patients requested by the professional to use denture adhesives sometimes feel that the treatment is lacking in quality and/or that the prosthesis is unacceptable. The early literature suggested that adhesives favored the continued use of ill-fitting dentures, enhanced alveolar bone resorption, increased the vertical dimension, favored hypersensitivity reactions, and affected the oral biofilms [1, 5–7]. More recently, controlled laboratory and clinical reports have contributed to a paradigm shift in opinions, suggesting that, in certain situations, clinical outcomes could be improved by the use of denture adhesives [8, 9] and that information on the correct use of denture adhesives should be part of post-placement care for patients [10]. Furthermore, the use of adhesives with correctly adapted dentures has been shown to improve stability and

retention, increase incisal bite force, minimize tissue discomfort, reduce the frequency of adjustments, and minimize the impaired blood supply to the mucosa [11–13]. Furthermore, adhesives also improve chewing efficacy and minimize the mucosal irritation and ulceration from ill-fitting prostheses [14, 15]. Finally, adhesives can also benefit xerostomic patients [16] and those with impaired muscular control [1].

As manufacturers aim to improve the clinical efficacy of denture adhesives, a range of products with different compositions and clinical presentations have been marketed. Adhesive materials can be categorized according to their solubility into soluble products—creams, pastes, and powders—and insoluble products—pads and wafers [17]. The active ingredients of soluble adhesives generally include polymer salts with short-term and long-term action, e.g., carboxymethylcellulose (CMC) and polyvinylether methyl cellulose (PVM-MA), respectively [18], which are expected to swell and become sticky when hydrated. Also included in soluble adhesives are several non-active compounds to facilitate administration (e.g., petrolatum and mineral oil, flavoring agents, dyes, and preservatives). The insoluble materials include both a component that becomes sticky in the presence of water and a fabric carrier [18]. Additionally, and in order to further improve retention, calcium salts and zinc have been added to several formulations. Insoluble adhesives include a laminated fabric web composed of woven napped material, a web of light polypropylene scrim or cellulose paper, with a water-activated component impregnated in the mesh, and sodium alginate or ethylene oxide may be added to increase adhesiveness [18].

Modifications in the compositions of adhesives have greatly improved biomechanical properties [18], but evaluations of the biocompatibility of adhesives have been lacking both in vitro or in vivo [19–22] despite clinical reports of suspected adverse reactions, microbial contamination [5, 22, 23], formaldehyde release, and low pH [24, 25]. One extreme example is of a zinc-containing denture cream which has been implicated in inducing hypocupremia and the development of serious neurologic consequences [26]. Thus, it is evident that the biological safety of denture adhesives in clinical application should be explored. This study aimed to contribute to the understanding of the cytotoxicity potential of a range of ten commercially available adhesives, following direct and indirect in vitro testing over fibroblastic cells.

Materials and methods

Denture adhesives tested

The commercial denture adhesives tested in this study are shown in Table 1. The products were tested in a direct assay, as a thick gel, and in the form of aqueous extracts.

Cytotoxicity assays with fibroblasts

Mouse fibroblast L929 cells (L-929, American Type Culture Collection) were cultured in α-Minimal Essential Medium (Gibco®) supplemented with 10% fetal bovine serum (Gibco®), 50 μg mL^{-1} ascorbic acid (Sigma-Aldrich®), 50 μg mL^{-1} gentamicin (Sigma-Aldrich®), and 2.5 μg mL^{-1} fungizone (Sigma-Aldrich®) at 37°C in a humidified atmosphere of 5% CO_2 in air.

For subculture, adherent cells were enzymatically released [0.05% trypsin (Sigma-Aldrich®), 0.25% EDTA (Sigma-Aldrich®)], and the cell suspension, cultured at 10^4 cells cm^{-2}, was used to perform the cytotoxicity assays.

Direct cytotoxicity assay

In the direct cytotoxicity assay, L929 fibroblasts were seeded over the surface of a thick gel of the different adhesives. Creams were prepared as a 5% (*w*/*v*) gel, and the powders and pad were prepared as a 2.5% (*w*/*v*) gel according to the methodology previously reported [22]. To prepare the gel, the appropriate amount of each adhesive (50 mg; 25 mg) was placed in the bottom of a 35-mm culture plate with 1 mL of culture medium, and the mixture was incubated for 12 h at 37°C in an atmosphere of 5% CO_2/air. In these conditions, all the adhesives formed a thick gel.

A suspension of L929 fibroblasts (1 mL; 4.5×10^4 cells mL^{-1}; 10^4 cells cm^{-2}) was seeded over the gel surface and cultured for 12 h. The controls were cell cultures performed in standard polystyrene culture plates.

Cultures were evaluated for cell viability using the MTT assay. At the end of the 12 h culture period, the pH of the culture medium was checked using a glass pH electrode and a pH meter (Mettler Toledo 340); the pH was around 7.0 for all the samples except for those contacting with Protefix® powder and Corega® cushions in which a pH of around 7.5 was observed.

Indirect cytotoxicity assays

In the indirect cytotoxicity evaluation, L929 fibroblasts were cultured in the presence of adhesive extracts prepared in static and dynamic conditions according to international standards [27, 28]. The appropriate amounts of each adhesive and complete culture medium were used to yield a 2% *w*/*v* extract (cream formulations) or a 1% extract (powders and pad formulation). The mixture was incubated at 37°C in an atmosphere of 5% CO_2/air for 12 h, in (1) static conditions and (2) under moderate continuous stirring. Thereafter, the extraction medium was filter-sterilized and tested undiluted and diluted. Adhesive cream formulations were tested as 2%, 1%, and 0.5% extracts. Powders and the pad formulations were tested as 1%, 0.5%, and 0.25% extracts.

Table 1 Manufacturers, lot number, and composition of the adhesives tested

Denture adhesive	Manufacturer	Lot number	Composition (according to manufacturer)
Corega® cream (adhesive 1)	Stafford Miller	R07341	Ca and Na salts of the copolymer PVM-MA, petrolatum, cellulose gum, paraffin
Protefix® cream (adhesive 2)	Queisser Pharma	020017	Ca and Na salts of the copolymer PVM-MA, CMC, paraffin, vaseline, silica, menthol, azorubin, *p*-hydroxybenzoic acid methyl esther
Steradent® cream (adhesive 3)	Reckitt Benckiser	S70231U008	Ca and Na salts of the copolymer PVM-MA, cellulose gum/HMC, cellulose gum/MC, paraffin, petrolatum, polyethylene oxide, mentha piperita, menthol, Cl 45430:1
Novafix® cream (adhesive 4)	Vitafarma	A 45	PVM-MA, CMC
Polident® cream (adhesive 5)	Stafford Miller	X08355A	Ca and Na salts of the copolymer PVM-MA, petrolatum, cellulose gum, paraffin, propyl paraben, flavoring agent, geraniol, linalool, Cl45430
Kukident® cream (adhesive 6)	Procter & Gamble	8167A	Ca and Zn salts of the copolymer PVM-MA, paraffin, cellulose gum, petrolatum, silica, menthol, peppermint powder, Cl 16185, Cl 75470
Aderyn® powder (adhesive 7)	Sociedade Farmacêutica Gestafarma	1171146	Karaya gum, flavoring agent
Corega® powder (adhesive 8)	Stafford Miller	N07181	Ca and Na salts of the copolymer PVM-MA, cellulose gum, flavoring agent
Protefix® powder (adhesive 9)	Queisser Pharma	018086	Na alginate, Na–Cu chlorophyllin, menthol
Corega® pad (adhesive 10)	Stafford Miller	635601	PEG-90M, microcrystalline wax, polybutene, cellulose gum

The pH of the extracts was checked using a glass pH electrode and a pH meter (Mettler Toledo 340) and found to remain at near-neutral values, except for the extracts from Protefix® powder and Corega® cushions (pH around 7.5). Higher extract concentrations were not tested as the liquid phase was totally incorporated by the adhesive with the formation of a gel.

L929 fibroblasts were plated in 96-well culture plates (100 μL; 3×10^4 cells mL^{-1}; 10^4 cells cm^{-2}) and cultured for 24 h to allow for cell attachment. Thereafter, the medium was removed and the extracts were added to the adherent cell layer. Cultures exposed to the extracts prepared in static conditions were cultured for 12 h. Cultures exposed to the extracts obtained under continuous stirring were cultured for 6, 12, 24, and 72 h. Cell cultures performed in standard polystyrene culture plates were used as control. Cultures were characterized for cell viability/proliferation by the MTT assay. In addition, cultures exposed for 12 h to the extracts prepared under stirring were stained for F-actin cytoskeleton and nucleus and observed by confocal laser scanning microscopy (CLSM).

Characterization of the cell behavior

Cell viability/proliferation

MTT assay Cell cultures were incubated with MTT [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (Sigma-Aldrich®), 0.5 mg mL^{-1}, during the last 3 h of the culture period tested. Viable cells reduce the MTT tetrazolium salt to a blue and insoluble product formazan, which precipitates in the cytoplasm. The formazan product was dissolved by adding DMSO (Sigma-Aldrich®), and the absorbance was measured at 600 nm in a microplate reader spectrometer (WS050 WellScan, Denley Instruments Ltd).

Immunofluorescent staining of F-actin cytoskeleton filaments and nuclei

Cultures exposed to the adhesive extracts prepared under stirring were fixed with 4% formaldehyde–methanol free (Sigma-Aldrich®), permeabilized with 0.1% Triton® (Sigma-Aldrich®), for 5 min at RT, and incubated in 10 mg mL^{-1} bovine serum albumin (BSA, 1 h, RT) (Sigma-Aldrich®) with 100 μg mL^{-1} RNAse (Sigma-Aldrich®). Following this, F-actin filaments were stained using 488 Alexa-Fluor-conjugated phalloidin® (Invitrogen®), 1:100, 1 h, RT, and nuclei were counterstained with 10 μg mL^{-1} propidium iodide (Invitrogen®), 10 min, RT. Fluorescence was visualized by CLSM.

Statistical analyses

Three independent experiments were performed. On the MTT assay, six replicates were set up at each condition. The effect of the denture adhesives on cell viability/proliferation is expressed as a percentage of control cultures (performed in standard polystyrene cell culture plates).

Statistical differences between control and the cultures exposed to the adhesives were analyzed by Student's *t* test. *P* values≤0.05 were considered significant.

Results

Culture of L929 fibroblasts over denture adhesive gels

Figure 1 presents the results regarding the cell viability/proliferation, evaluated by the MTT assay after L929 cells were plated over the surface of adhesives, as a 5% gel (creams) or a 2.5% gel (powders; pad), and cultured for 12 h. Dissolution of the formed formazan product and photometric evaluation allowed the quantification of the cell growth.

At these exposure conditions, adhesives 2 and 8 behaved similarly to controls (standard polystyrene plate). Adhesives 1, 3, 4, 5, 7, 9, and 10 had low cytotoxicity (a decrease in the cell growth <20%). However, adhesive 6 was highly cytotoxic (hardly supporting cell growth).

Exposure of L929 fibroblasts to denture adhesive extracts prepared in static conditions

Adhesives 1 and 2 had no effect on cell growth; adhesives 3, 4, 5, 8, 9, and 10 were toxic at the higher levels (about 20% decreased cell growth); and adhesives 6 and 7 had the highest toxicity potential, with 20–60% dose-dependent reduction on the cell growth, when adherent L929 cells were exposed for 12 h to the adhesive extracts prepared in static conditions (Fig. 2a).

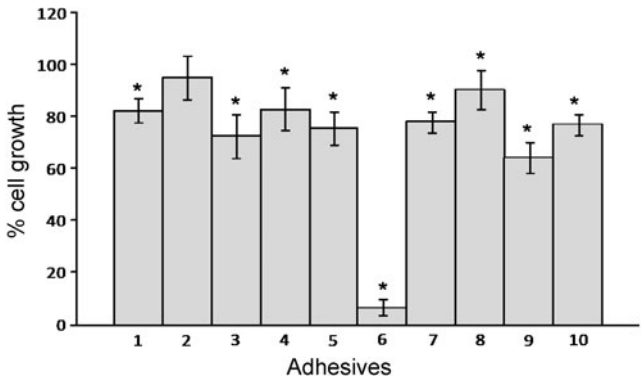


Fig. 1 Cell viability/proliferation (MTT assay) of L929 fibroblasts cultured over adhesive gels for 12 h (5% gels, cream formulations; 2.5% gels, powder and pad formulations). *Asterisks*, statistically different from control cultures performed in standard culture plates. Adhesives: Corega® cream (*1*), Protefix® cream (*2*), Steradent® cream (*3*), Novafix® cream (*4*), Polident® cream (*5*), Kukident® cream (*6*), Aderyn® powder (*7*), Corega® powder (*8*), Protefix® powder (*9*), Corega® pad (*10*)

Exposure of L929 fibroblasts to denture adhesive extracts prepared in dynamic conditions

Cell viability/proliferation

In general, the adhesive extracts caused dose-dependent inhibitory effects in the cell viability/proliferation when L929 cells were exposed for 6, 12, 24, and 72 h to the adhesive extracts prepared in dynamic conditions (under continuous stirring) (Fig. 2b). For the adhesive 1, 3, 4, and 10 extracts, significant deleterious effects were seen during the first hours of exposure, but later, cells were able to recover in the presence of the lower concentrations. In addition, for each tested concentration, cell viability/proliferation decreased with the time of exposure (6 to 72 h). This deleterious cumulative effect was less pronounced in cultures exposed to the extracts from adhesives 2, 4, and 8, i.e., only a slight decrease in cell viability/proliferation was observed throughout the culture time.

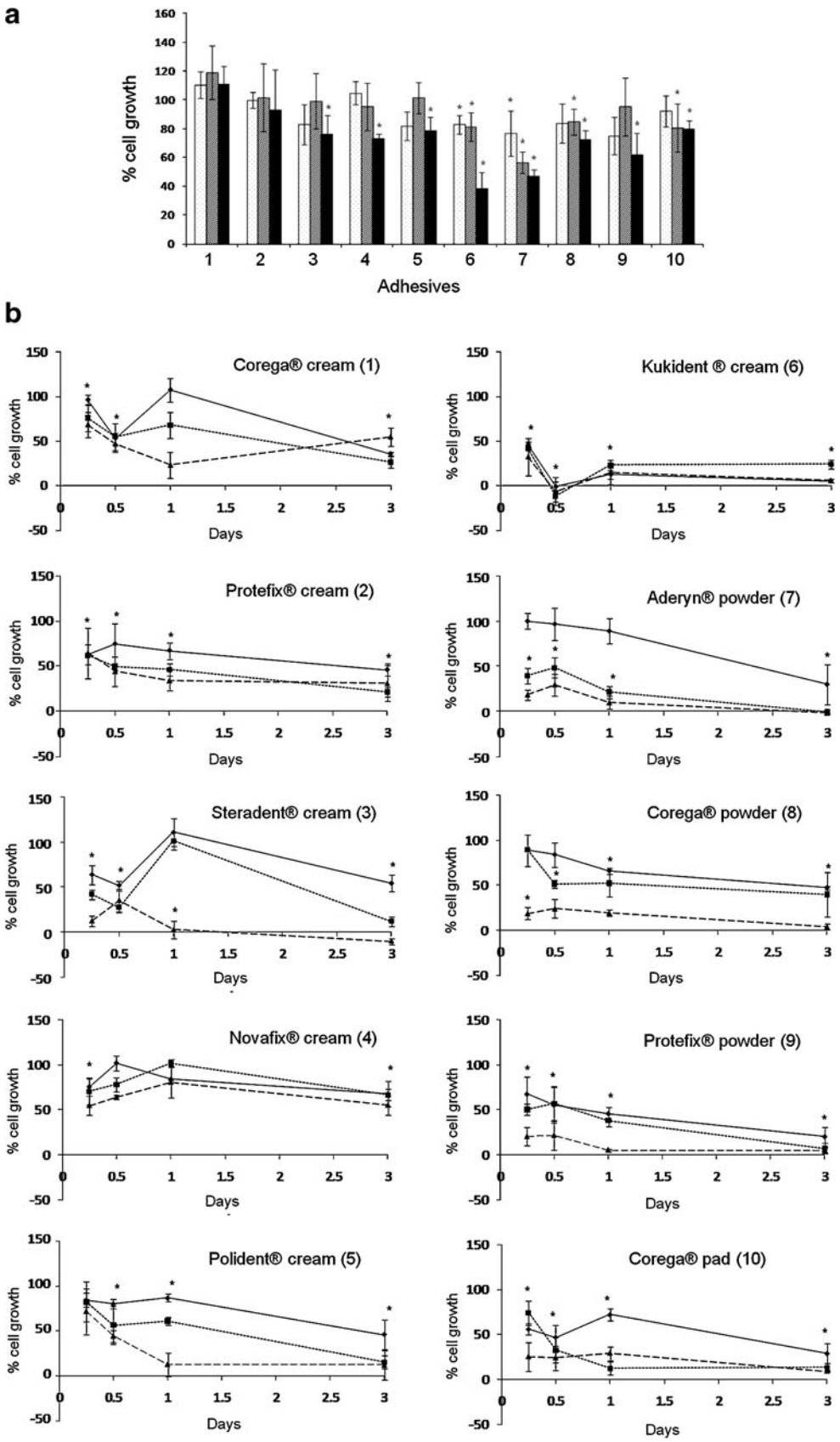
Adhesive 6 had the highest deleterious effect at the three extract concentrations tested and for longer times (≥12 h). On the other hand, extracts from adhesive 4 had only a low inhibitory effect on cell viability/proliferation at the three concentrations tested.

F-actin cytoskeleton organization

Control fibroblast cultures exposed for 12 h observed by CLSM after F-actin cytoskeleton and nucleus staining displayed well-spread cells with an elongated morphology and well-developed F-actin cytoskeleton and a prominent nucleus. The adhesive extracts prepared in dynamic conditions caused evident dose-dependent deleterious effects. Figure 3 shows representative images of the cultures exposed to 1% and 2% (creams) and 0.5% and 1% (powders; pad).

At the lower concentration tested, extracts from adhesives 3, 4, and 9 appeared to induce only minor

Fig. 2 **a** Cell viability/proliferation (MTT assay) of L929 fibroblasts exposed for 12 h to adhesive extracts prepared in static conditions. Cream formulations were tested as 0.5%, 1%, and 2% extracts (*w*/*v*); powder and pad formulations were tested as 0.25%, 0.5%, and 1% extracts (*w*/*v*). *Asterisks*, statistically different from control cultures performed in the absence of the adhesive extracts. Adhesives: Corega® cream (*1*), Protefix® cream (*2*), Steradent® cream (*3*), Novafix® cream (*4*), Polident® cream (*5*), Kukident® cream (*6*), Aderyn® powder (*7*), Corega® powder (*8*), Protefix® powder (*9*), Corega® pad (*10*). **b** Cell viability/proliferation (MTT assay) of L929 fibroblasts exposed for 6 to 72 h to adhesive extracts prepared in dynamic conditions. Cream formulations were tested as 0.5%, 1%, and 2% extracts (*w*/*v*); powder and pad formulations were tested as 0.25%, 0.5%, and 1% extracts (*w*/*v*). *Asterisks*, statistically different from control cultures performed in the absence of the adhesive extracts


a
% cell growth
Adhesives
b
Corega® cream (1)
Kukident ® cream (6)
Protefix® cream (2)
Aderyn® powder (7)
Steradent® cream (3)
Corega® powder (8)
Novafix® cream (4)
Protefix® powder (9)
Polident® cream (5)
Corega® pad (10)
% cell growth
Days

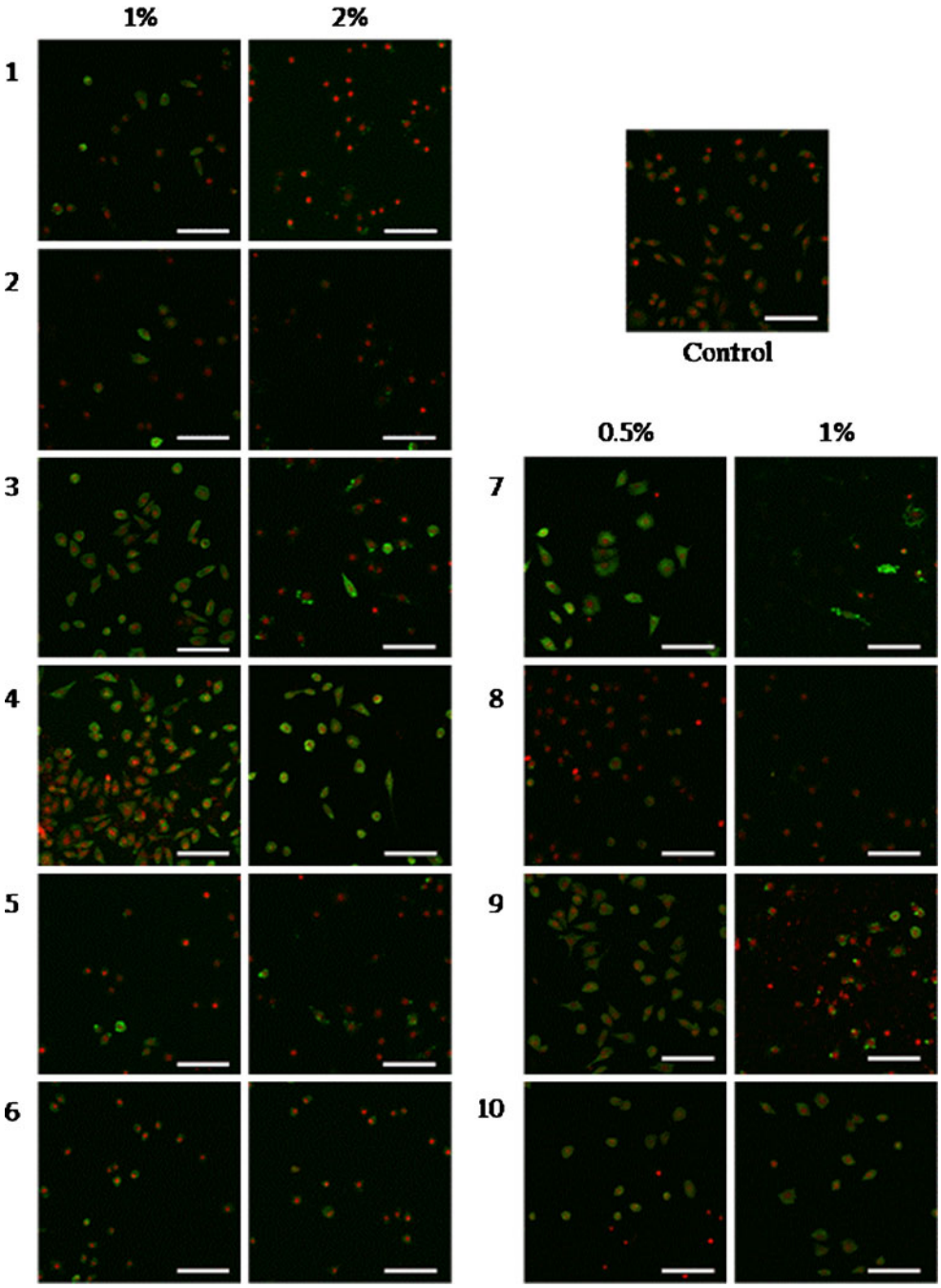


Fig. 3 Cellular morphology of L929 fibroblasts exposed for 12 h to adhesive extracts prepared in dynamic conditions (cream formulations, 1% and 2%; powder and pad formulations, 0.5% and 1%). CLSM images of cultures stained for F-actin (*green*) and nucleus (*red*). *Bar*: 100 μm. Adhesives: Corega® cream (*1*), Protefix® cream (*2*), Steradent® cream (*3*), Novafix® cream (*4*), Polident® cream (*5*), Kukident® cream (*6*), Aderyn® powder (*7*), Corega® powder (*8*), Protefix® powder (*9*), Corega® pad (*10*)

morphological changes. Extract from adhesive 7 caused an evident increase in the cytoplasmic volume although cells with condensed material were also present. Adhesives 1, 2, 5, 6, 8, and 10 caused condensation of nuclear material, a significant decrease in the cytoplasmic compartment associated with shrinkage of the actin cytoskeleton and a rounding up of the cells.

Exposure to higher adhesive extract levels caused a more significant cytotoxicity response. The above cellular alterations were more pronounced, and in addition, cellular disruption was observed in the presence of the extracts from adhesives 2, 3, 5, and 9. Reduction of the number of adherent cells was also seen in the presence of some extracts, especially those from adhesives 2, 5, 6, 7, and 8.

Discussion

Ten commercial denture adhesives were tested for the cytotoxic profile in direct and indirect in vitro assays. Most denture adhesives had some degree of cytotoxicity on the viability/proliferation of L929 fibroblast cells by 12 h of contact, the deleterious effects noted being dependent on the type of toxicity assay (direct/indirect), the concentration of the adhesive, and the duration of exposure. Direct contact of the L929 fibroblasts with the thick adhesive gels caused no, or only a slight, decrease in cell viability/proliferation, except for adhesive 6 which had a high cytotoxic effect. By 12 h of exposure, the adhesive extracts (especially those prepared in dynamic conditions) caused significantly higher growth inhibition of fibroblasts, which was dose dependent, and in addition, toxicity was cumulative throughout the 6–72-h exposure time.

Cytotoxicity of the adhesives was also evident upon the observation of the exposed cultures by CLSM. Dose-dependent effects on cell morphology, with evident disruption of the F-actin cytoskeleton organization, were seen in the presence of most adhesives. F-actin is a structural protein that is highly concentrated just beneath the plasma membrane, establishing an organized layer which controls cellular shape and surface movement, modulating cellular mechanisms subjacent to the proliferation and differentiation events [29]. Thus, cytoskeletal alterations such as those observed in the presence of the adhesive extracts may compromise cell growth and phenotype expression, considered an important sign of cytotoxicity response [29].

In the present work, the adhesives available as powders (adhesives 7, 8, and 9) and the insoluble pad (adhesive 10) were tested at levels half of those used in the cream formulations (adhesives 1 to 6), prepared as *w*/*v* mixtures (gels; extracts). This methodology was reported in a previous study [22], apparently to compensate for the absence of hydration of the dry formulations compared to the creams. However, the degree of hydration of the cream preparations is not known, and therefore, it is difficult to normalize the results regarding the adhesive formulations. This is not only between the creams and the dry formulations but also within the cream formulations, which most probably present significant differences on the water content. Therefore, the methodology used in the present work [22] is only a crude attempt to account for the absence of water content in the powders and the insoluble pad compared to the creams. The dry preparations were tested at levels half of those used in the creams assuming the medium value between 50% and 150% of the swelling ability reported for denture adhesive formulations [18]. In this way, direct and accurate comparison of the different adhesives regarding absolute cytotoxic effects is not possible, and thus, the aim of this work was mainly concerned with the establishment of a cytotoxic profile of the denture adhesive formulations.

Information regarding the cytotoxicity potentials of some of the adhesives tested in the present work was already available. Studies of several Corega® formulations and one Polident® formulation [19] and several Protefix® formulations [22] on L929 fibroblast cells have been reported. In the first study [19], the authors reported that the adhesives induced toxic effects, but details were not provided. In the second study [22], Protefix® formulations (cream, cushions, powder) assessed as 0.5–2% extracts prepared in static conditions (extraction time of 22–24 h) did not cause evident cytotoxicity after 24 h of exposure. In the present work, Protefix® cream (adhesive 2) and Protefix® powder (adhesive 9), tested as extracts prepared in static conditions, exhibited low cytotoxicity. However, comparison of the results presented here with previously reported studies [19–22] is difficult due to differences regarding the tested adhesives (or brands of the same adhesive), cell type and culture conditions, protocol used to prepare the adhesive extracts, and levels and exposure times.

Nevertheless, the present and previous studies of the commercially available denture adhesives do confirm varying degrees of cytotoxicity. Constituents leaching out and/or resulting from the breakdown of the adhesive blend appear to be the culpable agents. Identification of the culpable compounds is difficult in view of the fact that the composition and the concentration of adhesive constituents are rarely available in detail. In addition, cytotoxicity may also be caused by adhesive breakdown products. Moreover, two parameters to have in mind when assessing the cytotoxicity profile of specific compounds in vitro are the changes in the pH and osmolarity of the culture medium, which can exert a direct effect on cell proliferation, morphology, and functional activity. As reported, no significant alterations on the culture media pH were found, following the addition of the denture adhesives or adhesive extracts. On the other hand, many adhesive constituents, including salts and macromolecular fragments, might contribute to the increment of the osmolarity of the culture medium and directly influence the behavior of cultured cells. Literature reports have shown that L929 cells seem to be highly tolerant to osmolarity changes by accumulating organic “compensatory” compounds that seem to protect macromolecular structures from the disrupting effects of hyperosmotic media [30, 31]. Hypercondensation of the nuclear chromatin was established only when large abrupt hyperosmotic shocks were induced, in close association with a complete disappearance of DNA from the nucleoplasmic spaces [30], a situation that seems unlikely in the present work given the low adhesive concentrations tested. Moreover, eventual local osmolarity changes may be of minor relevance in clinical settings due to the diluting and

compensatory effects of the continuous contact with oral fluids.

Apart from this, some constituents included in the composition of some adhesives or present following the manufacturing process are reported to have deleterious effects [6, 19]. In the present work, Kukident® cream (adhesive 6) exhibited a high toxicity profile, yet the described composition is similar to that of the other adhesives, apart for some constituents provided as code numbers and zinc salts. Biocompatibility issues have been reported regarding the use of zinc-containing denture adhesives and the potential risk of developing neurologic diseases, in the setting of hypocupremia and hyperzincemia following the chronic use of large amounts of denture creams [26]. In vitro, the toxic effect of zinc salts has been previously studied and reported to exceed that of iron, copper, manganese, and cobalt in the same concentration range [32]. For L929 cells, the IC50 of $ZnCl_2$ salt was established to be 9.28×10^{-5} mol L^{-1}, and Zn was reported to be a highly toxic metal, of which the use within denture adhesives composition should be carefully appraised [33]. Accordingly, many manufacturers have changed the composition of their denture adhesives in order to exclude Zn from its constitution.

The true clinical significance of the in vitro observations reported here is difficult to ascertain. In clinical applications, any toxicity is liable to be lower than that in cultured cells due to several factors, including the tridimensional multilayered tissue organization with the cells being surrounded by abundant extracellular matrix and also because the oral mucosa is protected by several molecules and oral fluids, and is partially keratinized. The dynamic environment of the oral cavity, i.e., fluctuations of salivary flow, of temperature, and of pH, together with variable muscle movements, might as well influence cytotoxicity parameters.

Conclusion

The ten commercial denture adhesives tested in this study—six creams, three powders, and one insoluble pad—showed different degrees of cytotoxicity over L929 fibroblast cells but similar dose- and time-dependent cytotoxic profiles as assessed by direct and indirect assays. Thus, comparison of the cell behavior observed over the adhesive gel and in the presence of the extracts, prepared in static and dynamic conditions, showed that the thick polymerized gel presents low cytotoxic potential, but the extraction fluid containing products leaching out, and resulting from the adhesive breakdown, revealed higher cytotoxic effects on the assayed in vitro model.

Conflict of interest The authors declare that they have no conflict of interest.

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