Article

Biological effects of soft denture reline materials on L929 cells in vitro

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Abstract

Soft denture reline materials have been developed to help patients when their oral mucosa is damaged or affected due to ill-fitting dentures or post-implant surgery. Although reports have indicated that these materials leach monomers and other components that do affect their biocompatibility, there is little information on what cell molecules may be implicated in these material/tissue interactions. The biocompatibility of six soft liners (Ufi Gel P, Sofreliner S, Durabase Soft, Trusoft, Softone and Coe Comfort) was evaluated using a mouse fibroblast cell line, L929. Within 2 h of material disc preparation, each of the materials was exposed by direct contact to L929 cells for periods of 24 and 48 h. The effect of this interaction was assessed by alamarBlue assay (for cell survival). The expression of integrin $\alpha_5\beta_1$ and transforming growth factor β_1 was also assessed using plate assays such as enzyme-linked immunosorbent assay. Trusoft, Softone and Coe Comfort showed significantly reduced cell survival compared with the other soft lining materials at each incubation period. Furthermore, there were significant differences with these same materials in the expression of both integrin $\alpha_5\beta_1$ and transforming growth factor β_1. Soft liner materials may affect cell viability and cellular proteins that have important roles in wound healing and the preservation of cell viability and function in the presence of environmental challenges and stresses.

Keywords

Soft denture reline material, biocompatibility, fibroblast, integrin $\alpha_5\beta_1$, transforming growth factor β_1

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Introduction

The development of improved synthetic biomaterials for in vivo use is substantiated by the increasing demand for accelerated healing of tissues following trauma, disease or necessary surgical intervention. Regulatory testing of such materials is necessary before their application in patients and follows the normal pattern of in vitro testing which is performed prior to in vivo evaluation. Major developments in this field are occurring, with far more sensitive in vitro commercial plate assays available that provide quantifiable data, which is increasingly relevant to evaluating events occurring in vivo. However, many of the in vitro techniques still currently employed in dentistry for assessing biomaterials, such as dental polymers, and their components are simple and qualitative.[1–8] These techniques typically concentrate on whether cells are harmed in vitro

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or not with respect to cell number or basic cytotoxicity correlation[9] and rarely reflect the progress in our understanding of extra- and intracellular processes.

Soft denture reline products are compliant, viscoelastic materials used to reline all or part of the surface of a denture which interacts intimately with the oral mucosa tissues.[10] In essence, they serve to distribute the forces of mastication more evenly and to absorb energy. There are two types of denture soft lining polymers currently available, which differ depending on their composition: plasticized acrylic resin and silicone elastomers.[11] The reline material based on acrylic resin is composed of poly(ethyl methacrylate) (PEMA), monomer and plasticizers, and the materials based on silicone are essentially composed of polydimethylsiloxane.[11] Other soft materials developed are those that comprise tissue conditioners, which are composed of PEMA or similar polymers and the liquid contains no monomer,[11,12] only phthalates, plasticizers and alcohol.[3,10,13] Although widely used, these materials, as the silicone and acrylic resin-based types, have been associated with adverse symptoms including pain, blisters, ulcers, burning sensation and redness, and these have been attributed to the leached compounds from such materials.[10,14–17] This is particularly relevant as the reliners are placed in direct contact with wounded oral mucosa caused by ill-fitting dentures or subsequent to implant surgery.[11,18]

Tissue wound repair is complex and comprises proteins and processes involved with cell signalling, proliferation, migration, apoptosis and tissue remodelling.[19] In this context, integrins, present throughout the cell membrane, are pivotal molecules in the cell–cell and cell–extracellular matrix (ECM) communication, transmembrane connections to the cytoskeleton and activation of many intracellular signalling pathways.[20,21] There are 24 distinct integrins,[21,22] among them the $\alpha_5\beta_1$, which has been identified in keratinocytes and fibroblasts, participates in the re-epithelialization process during healing.[23,24] As one of the fibronectin receptors,[25] $\alpha_5\beta_1$ is highly expressed in fibroblasts and promotes their motility and proliferation.[26,27] During healing, fibroblasts need to be able to recognize the new proteins contained in the provisional ECM, which serves as a conduit for cell migration into the wound. To facilitate this, fibroblasts primarily use receptors of the integrin family.[28]

Studies have suggested that growth factors and their receptors are the key regulators of wound repair.[29,30] The integrins have been shown to regulate several growth factors, including transforming growth factor β (TGF-β), which is a pleiotropic cytokine that mediates a variety of cell functions, such as proliferation and differentiation in many cell types. There are three isoforms TGF-β_1, TGF-β_2 and TGF-β_3.[24,31] TGF-β is secreted in an inactive form which is activated at low pH or through the action of reactive oxygen species (ROS), proteases, thrombospondin 1 or several integrins.[24] It is also known that integrin $\alpha_5\beta_1$, expressed in re-epithelialization during wound healing, has been implicated in the upregulation of TGF-β expressed by keratinocytes and fibroblasts.[24]

It is known that cell physiology can be affected by various products leached from dental polymers,[1,5] and this may have an effect on integrin-mediated cellular function.[32] Given that both integrin $\alpha_5\beta_1$ and TGF-β_1 play important roles in wound repair,[21,30] and that soft reliners are used in direct contact with wounded oral mucosa, this article seeks to elucidate some biological in vitro effects of soft reline materials upon a cell line and, as far as the authors know, is the first that addresses this important topic. Thus, the hypothesis of this study was that the expression of both integrin $\alpha_5\beta_1$ and TGF-β_1 is increased in L929 cells in contact with six commercially available soft reline materials after short-term (24 or 48 h) in vitro exposure.

Materials and methods

In this study, tests were performed using direct contact between L929 cells and six soft denture reline materials, as detailed in Table 1. After two different periods (24 and 48 h), cell survival was evaluated by alamarBlue assay, a method that uses the indicator dye resazurin, which is dark blue in colour and possesses little intrinsic fluorescence until it is reduced by the metabolic activity of viable cells to resorufin, which is pink and highly fluorescent.[9] The expression of integrin $\alpha_5\beta_1$ and TGF-β_1 was also evaluated using enzyme-linked immunosorbent assay (ELISA) assays that according to the manufacturer (R&D Systems, Abingdon, UK) were designed to detect both phosphorylated and unphosphorylated integrin $\alpha_5\beta_1$ in cell lysates and to measure TGF-β_1 in acid-activated cell culture supernatant, respectively.

Material preparation

In total, 42 disc-shaped specimens of each material were prepared under aseptic conditions (18 specimens for alamarBlue and 12 specimens each for each of the ELISA assays). A stainless steel mould (14 × 1.2 mm^2) was used to fabricate the specimens. Each material was mixed according to the manufacturer's instructions before being placed in the mould upon an acetate sheet and a glass slab. Another acetate sheet and glass slab were placed over the material and light pressure was applied to remove excess material from the mould. The material was then allowed to polymerize or undergo gelation at room temperature. Prior to the biocompatibility tests, samples were sterilized by exposure to ultraviolet light for 20 min each side.

Cell culture

The L929 cells were obtained from ECACC, Porton Down, UK, and represent a connective tissue fibroblast cell line of

Table 1. Materials evaluated in this study..

Product	Type	Manufacturer	Powder/liquid ratio	Composition	Batch number	Polymerization/ gelation time at room temperature
Durabase Soft	Plasticized acrylic resin soft liner	Reliance Dental Mfg. Co., Alsip, IL, USA	1 g/0.83 mL	Powder – PEMA and benzoyl peroxide Liquid – MMA and DBP	29549	15 min
Trusoft	Plasticized acrylic resin soft liner	Bosworth Co, Skokie, IL, USA	1.06 g/1.15 mL	Powder – PEMA Liquid – benzyl butyl phthalate (plasticizer) and ethyl alcohol	0904-137	6 min
Ufi Gel P	Silicone-based soft liner	VOCO, Cuxhaven, Germany	Base and catalyst in a 1:1 ratio	Base – modified polydimethylsiloxane (A-silicone) Catalyst – platinum catalyst	1009051	10 min
Sofreliner S	Silicone-based soft liner	Tokuyama Dental Corp., Tokyo, Japan	Auto-dispensing system	Polyorganosiloxane Silicone resin powder Silica, amorphous	035E50	5 min
Softone	Tissue conditioner	Bosworth Co, Skokie, IL, USA	1.03 g/1.1 mL	Powder – PEMA Liquid – DPB and ethanol	0906-231	8 min
Coe Comfort	Tissue conditioner	GC America Inc, Alsip, IL, USA	1 g/0.83 mL	Powder – PEMA and zinc undecylenate Liquid – benzyl benzoate, cotton seed oil, ethanol, acetyl tributyl citrate, methyl salicylate, and peppermint oil	1006032	10 min

PEMA: poly(ethyl methacrylate); MMA: methyl methacrylate; DBP: di-butyl phthalate.

murine origin. Cells were cultured with Dulbecco's modified Eagle's medium (DMEM; Gibco, Paisley, UK) supplemented with 1% penicillin/streptomycin and 10% v/v fetal bovine serum (FBS; Gibco). The cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 and air. Media were changed every 3 days, and cells were passaged when approximately 80% confluent with 0.05% trypsin in 0.02% ethylenediaminetetraacetic acid (Sigma, Poole, UK).

Cells in direct contact with the materials

L929 were seeded at a density of 1×10^5 cells per well in 24-well plates (Corning, Inc., Corning, NY, USA) with 1 mL of culture medium and incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air for 24 h. After this time, the medium was removed, and the samples of each material were placed in the wells, under aseptic conditions, followed by 1 mL of DMEM supplemented with antibiotics only and not FBS. This procedure was undertaken to avoid the uncontrollable serum interaction and/or the neutralization of possible substances released by the materials during the incubation period.[2] Control cultures were selected as medium without serum in contact with the cells under the same culture conditions. The cells were in contact with the materials for 24 and 48 h, respectively, prior to collecting the cell culture supernatants, which were stored at −70°C until required for the ELISA assays.

Cell survival

Medium was removed and cells were washed with phosphate-buffered saline (PBS). To the samples (10% v/v of medium), alamarBlue was added, gently mixed and incubated for 3 h. Aliquots of 200 µL from each well were transferred to a black 96-well plate, and the fluorescence was measured using a Fluoroskan plate reader (Lab Systems, Loughborough, UK) at wavelength of 570 nm.

Integrin $\alpha_5\beta_1$-DuoSet IC ELISA (R&D Systems)

After removing the supernatant, the cells were washed twice with PBS, solubilized and the cell lysates were then stored at ≤ −70°C. Before starting the ELISA protocol, the samples were centrifuged at 2000× *g* for 5 min, and the supernatant was transferred to a clean Eppendorf Tube. The Capture Antibody (2.0 µg/mL in PBS without carrier protein) was added to coat a 96-well plate and incubated overnight at room temperature. After the aspiration/washing steps, the plate was blocked at room temperature for 2 h. The aspiration/wash step was repeated three times. Thus, 100 µL of test samples was added to the wells, and

after incubation for 2 h, Detection Antibody (150 ng/mL in IC Diluent), Streptavidin-HRP (diluted to the working concentration specified in IC Diluent) and Substrate Solution were added to each well, as recommended by the manufacturer. Finally, reaction was terminated adding the Stop Solution, and the optical density was immediately determined using a spectrophotometer (Tecan, Männedorf, Switzerland) microplate reader at wavelength of 450 nm and wavelength correction set to 540 nm.

Quantikine ELISA assay for TGF-β_1 (R&D Systems)

The cell culture supernatants from both time points were collected and immediately stored at ≤ 70°C. ELISA was performed according to the manufacturer's instructions. Briefly, the samples were activated using 100 µL of cell culture supernatant, followed by acidification (20 µL of 1 N HCl) for 10 min at room temperature, and neutralized by adding 20 µL of 1.2 N NaOH/0.5 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). All the reagents were acclimatized to room temperature; the unused microplate strips from the plate frame were removed, returned to the foil pouch and resealed. Then, 50 µL of assay Diluent and 50 µL of Standard, control or activated sample were added per well and incubated for 2 h at room temperature. Each well was then aspirated and washed four times. Subsequently, 100 µL of TGF conjugate was added to each well and incubated for 2 h. The aspiration/wash step was repeated. In sequence, Substrate Solution and Stop Solution were added to each well. Finally, 100 µL of Stop Solution and 100 µL of Substrate Solution were used as a Blank. The optical density was read at wavelength of 450 nm and wavelength correction set to 540 nm.

The alamarBlue assay was performed in triplicate on three separate occasions. Each of the ELISA assays was performed in triplicate on two separate occasions.

Statistical analysis

Values of absorbance obtained in the alamarBlue assay were normalized to the absorbance of the control cultures (medium without serum) and expressed as percentage survival. For both ELISA assays, standard curves were created using ReaderFit software (Hitachi Solutions America Ltd, San Bruno CA, USA) capable of generating a four-parameter logistic (4-PL) curve fit, and the concentration (pg/mL) of each protein in each sample tested was calculated. Data from alamarBlue assay and ELISA assays were tested for normality using the Shapiro–Wilk test. The data were then tested for significance using a Mixed Design two-way analysis of variance (ANOVA) for the alamarBlue data and Independent two-way ANOVA for the integrin and TGF data, respectively. The statistical analysis software used was SPSS v.17.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences between test groups were assessed using post hoc honest significant difference (HSD) Tukey's test. Statistical significance was considered at $p < 0.05$. For alamarBlue assay data, a comparison between the two periods of direct contact of the cells with the materials was conducted using 95% confidence intervals with Bonferroni correction ($\alpha = 0.0083$). For TGF assay data, post hoc analysis followed a simple effect approach and evaluated which material(s) contributed to the differences. This was performed in order to improve statistical power as well as to clarify interpretation of results.[33]

Results

AlamarBlue

The two-way ANOVA of alamarBlue assay showed significant effects for the factors material type, time in culture as well as the interaction between them ($p < 0.001$). Table 2 shows the percentage viability values relative to control wells without material. For both periods, the highest percentage of cell viability was observed for Sofreliner S, followed by Ufi Gel P and Trusoft, while the lowest means were obtained with materials Durabase Soft, Coe Comfort and Softone, which did not differ from one another (Table 2). For Sofreliner S, Trusoft, Durabase Soft and Coe Comfort, there were significant differences between the two periods, with higher mean values at 48 h.

Integrin $\alpha_5\beta_1$

The results of the two-way ANOVA of integrin data revealed that there were significant effects for material type ($p = 0.018$) and time in culture ($p < 0.001$), but not for their interaction ($p = 0.780$). Table 3 indicates a significant difference between Coe Comfort and Trusoft, but only Trusoft resulted in a mean integrin value (pg/mL) less than control. With regard to the time in culture, the mean protein concentration obtained after 48 h (7941 ± 594 pg/mL) of direct contact was significantly higher compared to 24 h (7273 ± 391 pg/mL) regardless of the material type.

TGF-β_1

The results of two-way ANOVA for TGF-β_1 data demonstrated that the factors material type and time in culture ($p < 0.001$), as well as their interaction ($p = 0.030$), showed significant effects. Table 4 shows the comparisons among materials, which were performed within each period. After 24 h in culture, there were no significant differences in TGF expression among materials. However, after 48 h in culture, the lowest expression was seen for Coe Comfort and the highest for Softone, both tissue conditioners.

Table 2. Percentage survival (mean ± SD) for the alamarBlue assay.

Materials	24 h	48 h	Difference (CI)
Ufi Gel P	76.3 (20.4)^{B}	78.1 (12.1)^{B}	1.8 (22.8 to −19.2)
Sofreliner S	97.2 (34.3)^{C}	141.1 (47.5)^{C}	43.9 (60.0 to 27.8)*
Trusoft	60.7 (9.1)^{B}	90.4 (21.8)^{B}	29.6 (47.9 to 11.3)*
Durabase Soft	6.2 (2.0)^{A}	8.2 (1.5)^{A}	2.0 (2.9 to 1.1)*
Coe Comfort	6.5 (2.0)^{A}	8.3 (1.5)^{A}	1.7 (2.8 to 0.6)*
Softone	8.1 (0.6)^{A}	11.2 (3.4)^{A}	3.2 (6.7 to −0.4)

CI: confidence interval; SD: standard deviation.
For each period, mean per cent values with the same letters (A, B and C) are not statistically different ($p > 0.05$). Those with different letter assignments exhibit a significant statistical difference.
*Significant difference for comparison between the two periods, using 95% CI with Bonferroni correction ($\alpha = 0.0083$).

Table 3. Mean values (±SD) for integrins for each material (in pg/mL).

Materials	24 h	48 h	HSD test*
Ufi Gel P	7293 (194)	7892 (353)	AB
Sofreliner S	7266 (391)	8072 (600)	AB
Trusoft	6914 (384)	7313 (521)	A
Durabase Soft	7182 (448)	7992 (513)	AB
Coe Comfort	7316 (414)	8236 (861)	B
Softone	7383 (318)	7917 (557)	AB
Medium without FBS	7697 (299)	8084 (442)	B

FBS (Control): fetal bovine serum.
* Regardless of each time period, mean per cent values with the same letters (A and B) are not statistically different ($p > 0.05$). Those with different letter assignments exhibit a significant statistical difference.

Table 4. Mean values (±SD) for TGF for each material (in pg/mL).

Materials	24 h	48 h
Ufi Gel P	2298 (239)^{A}	3008 (350)^{BC}
Sofreliner S	2126 (410)^{A}	2264 (806)^{AB}
Trusoft	1691 (147)^{A}	2111 (198)^{AB}
Durabase Soft	1426 (122)^{A}	2200 (644)^{AB}
Coe Comfort	1565 (316)^{A}	1751 (290)^{A}
Softone	2067 (642)^{A}	3625 (527)^{C}
Medium without FBS	2048 (507)^{A}	2006 (625)^{AB}

FBS (control): fetal bovine serum; TGF: transforming growth factor; SD: standard deviation.
For each period, mean per cent values with the same letters (A, B and C) are not statistically different ($p > 0.05$). Those with different letter assignments exhibit a significant statistical difference.

Discussion

Although denture soft lining polymers are widely used, some attention as to their clinical significance and use has been raised as they may cause adverse reactions to the oral tissue including ulceration and oedema.[14,16,17] These materials are also commonly used in areas of ulcerated tissue or healing phase wounded oral mucosa.[11,18] In addition, in the oral cavity, they are exposed to a complex variety of substances that may result in polymer degradation and component products leaching from the materials.[7] However, the impact of released compounds from denture soft lining polymers on the physiological processes of local cells and tissues is rarely reported in the literature, with studies often limited to cytotoxicity tests, that is, whether the cells 'live or die'.[1–6,8] Thus, in this study, the effect of soft reliners was evaluated not only on cell survival, using the alamarBlue cell metabolism assay, but also on two molecules that act as key regulators in wound repair, namely, Integrin $\alpha_5\beta_1$ and TGF-β_1, using ELISA 96-well assays. Unlike many other cell metabolism/survival assays commonly used, such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT),[1–6,8] alamarBlue is a non-destructive method[9] that allows continuous monitoring of the same cell population throughout the entire study period. The ELISA assays are based on specific immobilized antibodies that bind Integrin $\alpha_5\beta_1$ heterodimer and the growth factor TGF-β_1.

Given that the soft denture reline materials can come into contact with both epithelial and underlying exposed connective tissue cells, for this study, a fibroblast cell line was chosen, namely, L929 cells (ECACC). Although primary cells may more closely resemble in vivo physiology or conditions, they do not offer consistent results in vitro because of the issues of senescence as the cells are continually passaged. Moreover, primary cells derived from different patients can behave differently in culture conditions depending on the genetics and age of individuals from whom the tissue was derived. The L929 used in this study is a very conventional, widely used and established cell line, being an appropriate cell model for assessing the material effects.

The results showed that the two silicone materials (Ufi Gel P and Sofreliner S) were the least toxic. Despite the relative high standard deviation values shown by Sofreliner S, ANOVA demonstrated that this material induced the greatest metabolic response in L929 cells after 48 h compared to the control conditions. With regard to the high viability shown by Sofreliner S (value 141.1), this may have been due to the cartridge and dispenser system used

to mix and create the polymer prior to gelation. Although designed to prevent bubble formation, it could not be completely determined whether a reproducible disc was synthesized each time, and this may have partly explained the observed variable results. This was to be expected as these two materials are addition-polymerization silicones and do not comprise any of the more toxic acrylic components, such as monomers, or phthalates and esters of aromatic carboxylic acids that are used as plasticizers[1,3,10–17] in the remaining materials (Table 1). It has been observed that silicone-based soft liners released compounds at low concentrations levels[15] and did not significantly decrease L929 cell viability.[3,4] In addition, lower cytotoxicity was found for silicone-based soft liners compared with the acrylic-based materials,[8] as seen in this study. The two acrylic-based materials (Trusoft and Durabase Soft) induced less metabolic activity (60.7%–90.4% and 6.2%–8.2%, respectively) over the 48 h time in culture compared to the control cells. Both of these acrylic resins are phthalate-based, although Trusoft does not have a monomer phase, which may explain the greater cellular metabolic response compared to Durabase Soft. Furthermore, the two PEMA tissue conditioners (Softone and Coe Comfort) exhibited the least metabolic response by the cells with no more than 11.2% compared with the control rates after 48 h in culture. The leaching out of the plasticizer and ethyl alcohol components[10,13,34] and the probable enhancement of hydrolytic biodegradation by the absorption of water into the materials[7] could account for this marked decrease in alamarBlue reduction by the cells. Similar to the findings reported here, the exposure of L929 cells to the plasticizer di-*n*-butyl phthalate or to the methacrylic acid, a degradation product from methacrylate monomers, for 24 h also led to a reduction in cell metabolism and DNA synthesis.[5] The processes underlying the toxicity of monomers and plasticizers have not been fully elucidated. However, some mechanisms have been reported, such as DNA damage,[35] injury to the lipid bilayer,[36] changes in the mobilization of calcium ions (Ca^{2+}), which play an important role in many pathways of cellular signal transduction,[36] and oxidative stress due to the ROS generation.[35,37]

In this study, the cells were temporarily devoid of growth factors during 48 h in culture (due to lack of serum in the medium), as well as the adverse effects of the leachants on the cells together may have slightly increased the expression of integrin $\alpha_5\beta_1$ after 48 h compared to 24 h (Table 3). Integrin $\alpha_5\beta_1$ exhibits ligand specificity for fibronectin[25] and fibrin, important ECM glycoproteins, which play major roles in adhesion, migration, cell growth and differentiation and are vital for processes such as wound healing.[38] Cells that cannot adhere effectively to their substratum/ECM undergo apoptotic events far more readily than those cells denied growth factors.[39] Chemical insults, including many cytotoxic drugs, or leachants from dental materials and serum withdrawal can lead to mitochondrial release of cytochrome C.[40] Integrins have been shown to protect cell viability in response to stress and apoptotic stimuli, in particular to signals that activate the mitochondrial pathway. The ligation of integrin $\alpha_5\beta_1$ leads to increased expression of Bcl-2,[41] which is associated with apoptosis,[42] and increased resistance to serum withdrawal. Integrin $\alpha_5\beta_1$ has also been implicated in tumour angiogenesis but there is some controversy whether it is associated with tumour suppressive effects or it may have a promoter role.[43]

The results of this study also demonstrated that TGF-β_1 showed enhanced upregulation in cells maintained for 48 h compared with those maintained for 24 h in culture. In particular, Ufi Gel P, Durabase Soft and Softone showed marked increases from 24 to 48 h of 30%, 40% and 75%, respectively. The large difference in expression observed after 48 h in the tissue conditioners may be due to the very different compositions of Softone and Coe Comfort, respectively. The latter material has numerous compounds not present in Softone, which may have contributed to the decreased expression. Transforming growth factor-β (TGF-β) protein family consists of three cytokines (TGF-β_1, β_2, and β_3) with TGF-β_1 being the most abundant isoform in many tissues. TGF-β molecules are deemed to act as cellular switches that regulate a wide variety of cellular processes such as immune function, cell proliferation and epithelial–mesenchymal transition and hematopoiesis.[44]

The range of percentage increase of integrin protein observed was 8%–13% for the six materials from 24 to 48 h in culture, whereas for TGF-β_1, the range of percentage increase was 12%–75%. This suggests that TGF-β_1 protein expression was markedly increased over the culture period compared with integrin. Among the highest percentage increases after 48 h that were observed in the ELISA assays, Durabase Soft, Sofreliner S, Trusoft and Softone were deemed the materials that had the greatest effect on expression of both integrin and TGF-β_1. An increase in TGF-β_1, and also integrins, has been associated with the maturation of a proto-myofibroblast to a mature myofibroblast,[45,46] which is a characteristic of healing wounds. It has also been reported that increased levels of TGF-β_1 increases keratinocyte proliferation, which would be necessary in areas of oral mucosa devoid of a keratinized epithelium.

The absence of serum in the culture system described here suggests that the proteins TGF-β_1 and integrin $\alpha_5\beta_1$, as detected by the ELISA assays, must have been secreted by the cells and that the relative expressions of each protein were due to the leachants of the reline materials. However, it is important to note that this was an acute study only depicting cell activity over 48 h in culture. In addition, although in vitro tests such as those described in this study provide fundamental information for identifying

specific mechanisms of cellular response in the presence of materials as well as correlation of their clinical performance,[47] no matter how much control of the in vitro environment is imposed, the diverse and complex in vivo mechanisms can never be fully mirrored in vitro. Nonetheless, the results of this study have revealed, for the first time, that commercially available soft reline materials can trigger the expression of particular cellular proteins related to wound healing and other cell processes in the oral cavity.

Conclusion

The silicone-based materials, Ufi Gel P and Sofreliner S, promoted higher cell survival compared to the other soft liners and no change in the expression of both $\alpha_5\beta_1$ and TGF-β_1. Taken together, these results suggested that the silicone-based soft liners may have a more suitable biological behaviour and might reduce the risk of adverse effects during clinical use.

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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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