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L929 fibroblast bioassay on the in vitro toxicity of $SnCl_2$, H_3PO_4, Clearfil SE primer and combinations thereof

KEYWORDS
L929,
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trypan blue,
adhesive dentistry

SUMMARY
Stannous chloride ($SnCl_2$, 35%) can increase microtensile bond strength between a self-etching adhesive and dental hard tissue either alone or in combination with phosphoric acid (H_3PO_4, 35%). Whereas cell toxicity of H_3PO_4 has been sufficiently investigated, little is known about the toxicity of concentrated $SnCl_2$. The present study determined the in vitro toxicity of $SnCl_2$, H_3PO_4, the primer of a self-etching adhesive (Clearfil SE) and combinations thereof at three concentrations (0.01%–0.3%) in a L929 fibroblast bioassay. Cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, the integrity of cell membranes was visualised by trypan blue staining (cell necrosis assay). Cell viability was impaired at concentrations between 0.09% and 0.13% for $SnCl_2$, between 0.06% and 0.09% for H_3PO_4, and between 0.19% and 0.29% for Clearfil SE primer. Combinations of agents showed additive toxic effects. $SnCl_2$ showed a slightly lower but comparable in vitro toxicity to H_3PO_4, which gives a perspective for further in vitro, in situ and clinical studies on this issue, and for the intraoral use of $SnCl_2$ to increase bond strength between an adhesive system and both enamel and dentine.

Introduction

Numerous adhesive systems have been developed to durably anchor composites to dental hard tissues. The established products are total etch adhesives, which use concentrated phosphoric acid (H_3PO_4) as etching agent (pH around zero) prior to application of the adhesive components. More recently, self-etching systems have been developed, which use acidic primers with a pH around 2 instead of H_3PO_4. Both have advantages and disadvantages: etching with H_3PO_4 results in high values of bond strength to enamel (Pivetta et al. 2008) as it causes a distinct retentive etching pattern (Johnston et al. 1996). In dentine it easily decalcifies the dentinal collagen, but a major flaw of total etch procedures is that the infiltration of the demineralised dentine matrix with the adhesive material may be incomplete, particularly after over-etching, which reduces bond strength (Hashimoto et al. 2002). Consequently, total etching techniques require different etching times for enamel and dentine, which is difficult to achieve in clinical practice.

Self-etching adhesives deal with these aspects by simultaneous demineralisation and infiltration of the dentine by acidic primers with relatively mild pH. A limitation of these systems, however, is that enamel etching is weak (Lima et al. 2012) and, as a consequence, bond strength values are much lower than those obtained with total etch systems (Schlueter et al. 2013). Pretreatment with phosphoric acid improves bond strength of self-etching adhesives to enamel, but has the above-mentioned possible unfavourable effects on bonding to dentine (Van Landuyt et al. 2006).

A potential solution in this context is pretreatment with stannous chloride ($SnCl_2$), which led to a distinct improvement of enamel bond strength (Schlueter et al. 2013) and even some increase in bond strength to dentine (Peutzfeldt et al. 2013) when applied as a concentrated solution (35%, pH around zero) prior to use of a self-etching adhesive system (Clearfil SE; Kuraray, Okayama, Japan).

Given these promising findings, it seems worthwhile to investigate whether such concentrated, acidic $SnCl_2$ solutions carry the risk of toxicity or side effects. The toxicity of the gold standard H_3PO_4 has been sufficiently investigated (for example Kinomoto et al. 2003). Even though $SnCl_2$ in general is classified as a low-risk agent (Winship 1988; European Food Safety Authority [EFSA] 2005; World Health Organization 2005) and is widely used in dentistry at a low concentration (Muhler 1958; Clark et al. 1988; Paraskevas & van der Weijden 2006; Mallatt et al. 2007; Ganss et al. 2010; Sharma et al. 2010), little is known about its cytotoxicity at higher concentrations (Reinhardt et al. 1985; Viau et al. 2009). Moreover, $SnCl_2$, H_3PO_4 and the primer of a self-etching adhesive system (Clearfil SE) may potentially be used in combination, but no information exists on their combined cytotoxicity.

The present study determined, therefore, the toxicity of H_3PO_4 and the primer of a self-etching adhesive (Clearfil SE), which are standard agents in adhesive dentistry, and of $SnCl_2$ as a potential new agent for adhesive dentistry. Additionally, the toxicity of combinations of the three agents at three concentrations was investigated. The L929 fibroblast bioassay was used for testing. This is a standard assay (ISO 10993 [U.S. Food and Drug Administration 2013]) for evaluating the biocompatibility of a medical device prior to clinical studies, and has already been used to investigate the toxicity of dentine bonding agents, including those of the Clearfil series (Koulaouzidou et al. 2009; Kusdemir et al. 2011).

Materials and Methods

Cell culture

All tests were performed with the L929 mouse skin fibroblast cell line (kindly provided by Martin Willheim, Center of Physiology, Pathophysiology and Immunology, Medical University of Vienna, Austria). The cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco-Life Technologies, Gaithersburg, MD, USA) supplemented with 10% foetal calf serum (FCS; Gibco-Life Technologies), penicillin (100 units/ml) and streptomycin (100 µg/ml; Gibco-Life Technologies). Cells were incubated at 37°C in a humidified incubator at 5% CO_2. Confluent cells were treated with 0.5% trypsin solution for detachment, diluted with 10% serum-containing medium. The cells were then plated in growth medium at 30,000 cells/cm² into 96 well culture plates. Culture plates were incubated for 24 h at 37°C in a humidified incubator at 5% carbon dioxide (CO_2) prior to stimulation of the cells with the test materials.

Test materials

The cytotoxicity of various dilutions and combinations of $SnCl_2$ (provided by GABA International AG, Therwil, Switzerland), H_3PO_4 (ortho-phosphoric acid 85%, 79623; Sigma Aldrich, Seelze, Germany) and the primer of Clearfil SE (CF; Kuraray; LOT number 041867) were investigated. Dilutions were prepared with serum-free medium (DMEM) from a 35% $SnCl_2$ and a 35% H_3PO_4 stock solution with native pH (approximately zero); the primer of CF was used as the stock solution and its concentration was set at 100%.

Two dilution series of the components were tested. The first series was used to narrow down the concentration range in which a decrease of absorption to one half of the maximum (half maximum of toxicity = half maximal cell viability; $A_{Max/2}$) can be expected (data not shown). The second dilution series was used to define the half maximum of toxicity more precisely. A starting concentration for $SnCl_2$ and H_3PO_4 of 1.5% and for the primer of Clearfil SE of 16.7% was used according to the approximated span for the toxicity threshold found in the first dilution series. Dilution steps of 1:1.5 were used. For $SnCl_2$ and H_3PO_4, twelve different concentrations were tested and for the primer of Clearfil SE, 24 different concentrations were investigated. A total of 100 µl from each concentration of each tested solution was added to each well containing the L929 cells.

The effects on cell viability of combinations of the components in comparison to those of the components on their own were investigated at three concentration levels (low, medium and high: 0.01%, 0.03% and 0.1% for both $SnCl_2$ and H_3PO_4, and 0.03%, 0.1% and 0.3% for the primer of Clearfil SE). The "high" concentration chosen corresponded to the half maximum of toxicity and can, therefore, provoke toxic effects on its own. The "medium" and "low" concentrations show only minor toxicity on their own but might provoke toxic effects in combinations with other agents. Fresh serum-free medium without any additives served as negative control; undiluted 35% phosphoric acid served as positive control. After stimulation, culture plates were again incubated for 24 h at 37°C in a humidified incubator at 5% CO_2.

MTT conversion assay

To measure the viability of cells, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (MTT; 0.5 mg/ml; M5655; Sigma, St Louis, MO, USA [Mosmann 1983]) was added to the wells and the cells were incubated for 2 h at 37°C in a humidified incubator at 5% CO_2. Cells were examined by light microscopy after the 2 h incubation time at a 10-fold magnification (Leica DM IL; Leica Microsystems GmbH, Wetzlar, Germany). The MTT solution was then removed and the formazan crystals were dissolved with dimethyl sulfoxide (Sigma Aldrich, Seelze, Germany). The absorbance (A) was determined spectrophotometrically (ELX 808; Bio-Tek Instruments Inc., Winooski, VT, USA) at 550 nm. All assays were performed in duplicate in the same experiment. Measurements of absorbance after MTT assays were performed for the dilution series only. Microscopic examinations after MTT incubation were performed for the dilutions series as well as for the combinations of the three components under investigation.

Trypan blue staining

Necrosis was assessed by light microscopic examination at a 10-fold magnification (Leica DM IL) after trypan blue staining (0.1%, 15 µl per well). Trypan blue staining (Evans & Schule-

MANN 1914) was performed for combinations of the three components under investigation for the single components at the three concentrations.

Data analysis

Absorbance values as well as micrographs were analysed descriptively. For determination of the half maximum of toxicity, the first dilution series was used to localise the concentration range (data not shown). A more precise determination was performed with the second dilution series. The half maximum of toxicity was defined as the half maximum absorption ($A_{Max/2}$). Maximum absorbance (A_{Max}) was defined as the absorbance at the lowest concentration used in each dilution series, the minimum absorbance (A_{Min}) was the lowest value measured for each agent in the dilution series. $A_{Max/2}$ was calculated by the following formula: $A_{Max/2} = (A_{Max} - A_{Min})/2 + A_{Min}$

Results

Absorbance of MTT reduction products

Plots of absorbance values for the second dilution series of the single components are shown in figures 1a to 1c. Half maximum absorption ($A_{Max/2}$) in the first dilution series was between 1.94% and 0.07% for $SnCl_2$, between 0.65% and 0.07% for H_3PO_4 and between 0.62% and 0.008% for the primer of Clearfil SE (data not shown). This indicated that the half maximum of toxicity can be found in this range. In the second dilution series, $A_{Max/2}$ was found to be between 0.09% and 0.13% for $SnCl_2$ (fig. 1a) and between 0.06% and 0.09% for H_3PO_4 (fig. 1b). For the primer of Clearfil SE, $A_{Max/2}$ was between 0.19% and 0.29% (fig. 1c).

Light microscopy of MTT reduction products (formazan crystals)

In order to assess cell morphology, micrographs were taken after incubation of the single components ($SnCl_2$, H_3PO_4 and primer of Clearfil SE), and combinations thereof, at the three different concentrations after MTT incubation. These are shown in figures 2a and 2b. A reaction between MTT and $SnCl_2$ was seen at various concentrations, leading to background staining. Independent of the background staining, a clear intracellular formation of formazan crystals was visible under the microscope (fig. 2b); thus, cells were able to convert MTT into formazan crystals, indicating that L929 cells were viable.

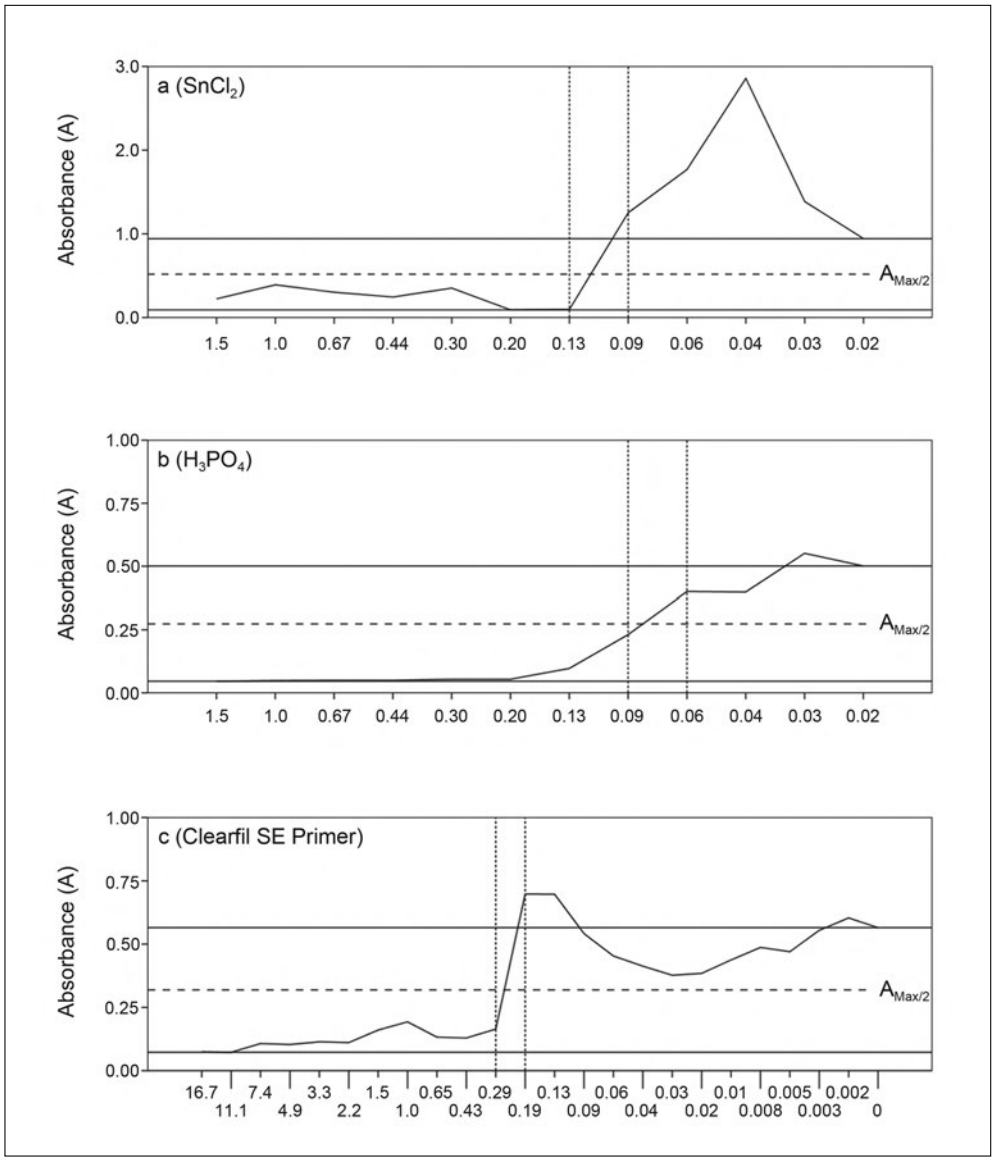

Fig. 1 Results of the spectrophotometrically determined absorbance (A) values of the second dilution series. The concentrations investigated are displayed on the x-axis. The half maximum of toxicity or half maximum reduction of cell viability was defined in the case that the absorbance value reaches the half maximum absorbance ($A_{Max/2}$; horizontal dashed lines). Maximum absorbance (A_{Max}) was defined as the absorbance at the lowest concentration in each dilution series (upper solid line; lower solid line = minimum absorbance). $A_{Max/2}$ was reached in the second dilution series for $SnCl_2$, between 0.09% and 0.13% (a), for H_3PO_4 between 0.09% and 0.06% (b) and for Clearfil SE Primer between 0.19% and 0.29% (c). The ranges in which $A_{Max/2}$ was reached are marked by the dotted vertical lines. All assays were performed in duplicate in the same experiment.

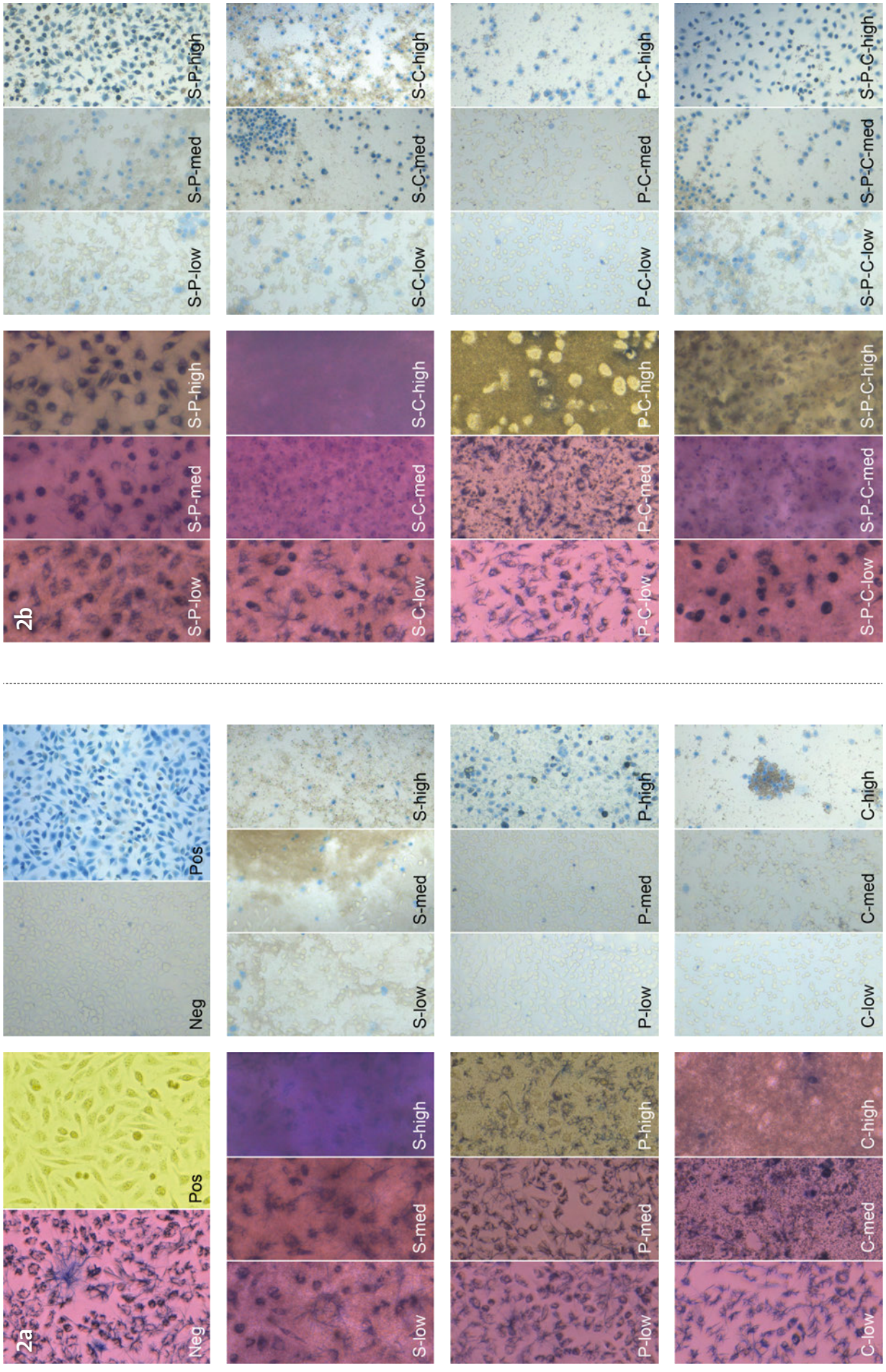

Fig. 2 Micrographs of L929 fibroblasts in the MTT and trypan blue assays after incubation with (a) the single components $SnCl_2$ (S), H_3PO_4 (P) and Clearfil SE primer (C) and (b) the combinations thereof at low, medium (med) and high concentrations. The left-hand columns show the results of the MTT assay, the right-hand ones of the trypan blue staining. The first row in (a) shows the reaction in the control groups; the negative control is on the left-hand side and the positive control is on the right-hand side. No discrepancy for either assay was found for the single components (a). However, for the combinations of $SnCl_2$ and H_3PO_4 at the high concentration, trypan blue staining was seen, indicating cell necrosis. Also, a concomitant formation of formazan crystals was detected, indicating viability of cells.

The MTT assay at the low concentration showed no reduction of cell viability for the single components or for combinations thereof. At the medium concentration, only the single components showed no effect on viability, whereas the combinations partially decreased it, in particular the combinations with the Clearfil SE primer. At the high concentration, the incubation of the single components resulted in clear toxicity for the Clearfil SE primer and slight toxicity for $SnCl_2$ and H_3PO_4. However, there was a tendency for fewer formazan crystals to be formed after H_3PO_4 incubation than after $SnCl_2$ incubation, confirming the results of the dilution series. The combinations of the components led to a reduction of viability in all cases, except for the combinations of $SnCl_2$ and H_3PO_4.

Light microscopy of trypan blue staining

After trypan blue staining, detachment of cells was observed after incubation with the Clearfil SE primer at the high concentration and after incubation with the Clearfil SE primer in combination with H_3PO_4. The micrographs had the expected appearance at the low and medium concentrations. The decrease in cell viability found in the MTT assay corresponded quite well with an increase in staining by trypan blue, indicating necrosis. At the high concentration, however, the combination of $SnCl_2$ and H_3PO_4 led to an increase in trypan blue staining without a decrease in cell viability measured with MTT. Comparable results were found for the combination of all three components, $SnCl_2$, H_3PO_4 and primer Clearfil SE primer.

Discussion

Phosphoric acid (H_3PO_4) is widely used in dentistry as a pretreatment agent for conditioning the dental hard tissue prior to insertion of dental fillings, without causing any permanent problems or alterations in the oral cavity if applied in conformity with recommendations for use. Therefore, it appears meaningful and appropriate to compare its behaviour with that of a new or alternative pretreatment agent.

The main finding of this study was that $SnCl_2$ showed a similar effect on cell viability to that of H_3PO_4. The half maximum of toxicity ($A_{Max/2}$) found in the present study for both $SnCl_2$ and H_3PO_4 was in a similar range – around 0.1% – and was around 0.25% for Clearfil SE primer. These results are in clear agreement with the literature, which reports a half maximal reduction of cell viability at 0.19% for $SnCl_2$ (24 h in vitro incubation of hamster fibroblast cell line) (Reinhardt et al. 1985) and at 0.3% for Clearfil SE primer (24 h in vitro incubation of transfected human pulp-derived cells) (Demirci et al. 2008), and cytotoxic effects for H_3PO_4 at concentrations of 0.2% (24 h in vitro incubation of human periodontal ligament cells) (Kinomoto et al. 2003). In all the studies cited as well as in the present one, the cells were directly incubated with the compound for a period of time, a situation which normally does not occur in clinical use and hence the results may not be directly transferable to the oral cavity. Therefore, an analysis of the acute toxicity could provide more germane information. However, in this context, some older studies regarding the potential intraoral use of the 35% $SnCl_2$ solution might be relevant. In an earlier investigation, the pulpal reaction after application of 10% and 30% stannous fluoride solutions on freshly prepared dentine in rats was tested. Little to no reaction of the pulp cells was provoked (Massler & Evans 1967; Myers et al. 1971), indicating that stannous ion-containing solutions, even at a high concentration of 35%, might be an option for intraoral use, for example to increase the microtensile bond strength (μTBS) of certain self-etching adhesives.

To the best of the authors' knowledge, the effect on cell viability of the combinations of the different agents has not yet been investigated. However, these agents are commonly used in combination (e.g. for anchoring a filling on dental hard tissue). Although the use of mouse skin fibroblasts only partially reflects the clinical situation, this well-established bioassay provides the first information on this issue. The concentrations investigated were chosen according to the determined half maximum of toxicity to ensure that in two cases (low and medium concentrations) the concentrations show only minor effects on cell viability on their own. The results obtained for the single components were as expected. For the combinations at the medium and high concentrations, an additive effect on cell viability was observed. In particular, the combinations with Clearfil SE primer increased these effects distinctly. Regarding the clinical relevance of these results, thorough rinsing should be performed after application of the concentrated $SnCl_2$ or H_3PO_4 solution in order to reduce potential toxic effects.

In addition to the results regarding the toxicity of the tested agents, an interesting reaction between the cells and the incubation agents was observed in the present study. The microscopic results of the MTT and trypan blue were complementary for all incubations except for combinations of $SnCl_2$ and H_3PO_4 at medium and high concentrations. Here, an increase in membrane permeability to trypan blue was found, indicating cell necrosis; however, in the MTT assay formation of formazan crystals was clearly visible. The microscopic findings showed that the cells were not damaged since they were neither detached from the bottom of the well nor were changes in cell morphology found. Another mode of action, which increases the permeability of the cell membrane without destroying it, must therefore be the reason for the trypan blue staining. It was reported in another study that incubation of neuronal cells with $SnCl_2$ led to a change in membrane potential (Gyori et al. 2000). The authors speculated that such a change might be a result of membrane channels opening through the direct interaction of stannous ions with channel proteins (Gyori et al. 2000). Therefore, it could be hypothesised that in the present study such interactions between the stannous ions and proteins of the cell membrane also occurred and that an opening of membrane channels led to an influx of trypan blue. As the concomitant trypan blue staining and formazan crystal formation occurred only after the combined incubation with $SnCl_2$ and H_3PO_4, it appears that phosphate ions are necessary for the hypothesised reaction to occur.

In future studies, the toxicity of the single components and of their combinations should be investigated in more detail. Not only the effect of a 24 h incubation but also the acute toxicity should be investigated. In addition, the fibroblast cell line used in the present study gives only a first insight into possible toxic effects. These mouse cells, however, might react differently to human cells. Therefore, for future studies other cells, preferably human cell lines from the oral cavity, should be used to perform in vivo studies investigating concentrated stannous ion-containing preparations in clinical applications. In addition, our findings should be confirmed by other methods such as propidium iodide staining to reinforce the results of the trypan blue staining. More information should also be gained about the potential of stannous ions to impact either the results of the MTT assay or of the trypan blue staining. Studies assessing the influx

of stannous ions into the cell in the presence of phosphate ions might be helpful.

In summary, the L929 fibroblast assay provided the first indications that the 35% $SnCl_2$ solution used shows toxicity comparable to that of the commonly used H_3PO_4. However, some additive effects on cell viability were found for this compound at higher concentrations, and in particular in combination with the Clearfil SE primer. Thorough rinsing prior to primer application might reduce potential toxic effects. The data provide the basis for further investigation of $SnCl_2$ solutions using intraoral cell lines with the objective of planning clinical studies to evaluate the effect of these solutions in the oral cavity (e.g. as a pretreatment agent to increase the μTBS of an adhesive system).

Résumé

Introduction

Il existe différents systèmes adhésifs avec des fonctionnements variables pour la liaison d'un composite avec les tissus durs dentaires. Une technique bien éprouvée est le «total-etch» qui consiste en un conditionnement des substrats dentaires durs à l'acide phosphorique (H_3PO_4) ou avec des systèmes self-etch. Le «total-etch» à l'H_3PO_4 produit une bonne liaison avec l'émail, mais l'acide peut surmordancer la dentine et diminuer sa capacité de collage, tandis que les systèmes self-etch permettent un bon collage dentinaire mais l'adhésion à l'émail n'est pas toujours optimale. Le collage à l'émail pourrait être amélioré en combinant un mordançage à l'H_3PO_4 sur l'émail avec un self-etch dentinaire, mais peut avoir potentiellement des effets négatifs sur l'adhésion dentinaire et n'est donc recommandé qu'avec des restrictions. Pour ces raisons, il serait souhaitable d'utiliser un conditionneur capable d'améliorer le collage à l'émail sans diminuer l'adhésion dentinaire. L'application d'une solution de chlorure d'étain ($SnCl_2$, pH = proche de 0) seule ou en combinaison avec l'H_3PO_4 serait une option. Les premières études ont montré que le $SnCl_2$ améliore nettement la liaison d'un self-adhésif avec l'émail ainsi qu'un peu celle à la dentine. Le $SnCl_2$ en faible dose ne représente pas un risque et est déjà utilisé régulièrement en médecine dentaire. Toutefois, lors de concentrations importantes (pH très acide), le $SnCl_2$ peut être potentiellement toxique, similaire à l'H_3PO_4. La toxicité de l'acide phosphorique a déjà été largement étudiée mais pas celle d'une forte concentration de $SnCl_2$ ou d'un de ces composants au niveau cellulaire. Ainsi, cette étude a testé in vitro (bioassay L929 de fibroblastes) la toxicité cellulaire de diverses concentrations de $SnCl_2$ en comparaison au H_3PO_4 et de celle d'un primer d'un self-adhésif (Clearfil SE), de même que la combinaison des trois agents en trois concentrations (faible, moyenne et forte; 0,01–0,03%).

Matériel et Méthodes

La vitalité cellulaire a été étudiée en utilisant un test MTT avec des mesures de la densité optique de cellules vivantes (couleur violette). L'intégrité de la membrane cellulaire de même que la morphologie cellulaire ont été révélées au bleu de Trypan (colore sélectivement les tissus ou cellules mortes). Les expériences ont été répétées deux fois.

Résultats et discussion

La vitalité cellulaire demi-maxima était trouvée pour une concentration de $SnCl_2$ entre 0,13% et 0,09%, pour l'H_3PO_4 entre 0,09% et 0,06%, et pour le Clearfil SE primer entre 0,29% et 0,19%. La combinaison des agents individuels a montré une augmentation de la toxicité lors d'un effet additif, surtout lors d'une combinaison avec le primer. $SnCl_2$ et H_3PO_4 étaient similaires dans leur toxicité cellulaire ainsi que leur effet sur la perméabilité membranaire. Ces résultats ont montré que les solutions concentrées de $SnCl_2$ peuvent a priori être utilisées cliniquement, mais devraient être confirmées par d'autres études intraorales sur des lignées cellulaires. Les connaissances provenant de cette étude devraient ouvrir de nouvelles perspectives d'utilisation de hautes concentrations de préparations à base d'étain dans le cadre de la technique adhésive in situ et en recherche clinique.

Zusammenfassung

Einleitung

Für den Verbund von Komposit und Zahnhartsubstanzen stehen zahlreiche Adhäsivsysteme unterschiedlicher Funktionsweise zur Verfügung. Ein bewährtes Verfahren in diesem Zusammenhang ist die sogenannte «Total-Etch»-Technik, die eine Konditionierung der Zahnhartsubstanzen mit Phosphorsäure (H_3PO_4) sowie selbstkonditionierende Systeme beinhaltet. Während Total-Etch-Systeme einen guten Verbund zum Schmelz erzeugen, aber mitunter Schwächen im Verbund zum Dentin durch Überätzung mit Phosphorsäure zeigen, erreichen selbstkonditionierende Präparate im Dentin gute Haftkraftwerte, der Verbund zum Schmelz ist jedoch nicht immer optimal. Die Schmelzhaftung selbstkonditionierender Systeme kann durch Vorbehandlung mit H_3PO_4 zwar verbessert werden, was jedoch mit potenziellen negativen Effekten in Bezug auf die Dentinhaftung erkauft wird und daher nur mit Einschränkungen zu empfehlen ist. Daher wäre eine Vorbehandlung wünschenswert, die den Verbund zum Schmelz verbessert, die Haftwerte zum Dentin jedoch nicht verringert oder idealerweise sogar erhöht. Ein vielversprechender Ansatz dazu ist die Applikation konzentrierter (35%) Zinnchloridlösungen ($SnCl_2$, pH-Wert nahe null) entweder allein oder in Kombination mit H_3PO_4. Erste Studien haben gezeigt, dass $SnCl_2$ den Verbund eines selbstkonditionierenden Adhäsivs zum Schmelz deutlich verbessert und darüber hinaus auch die Werte der Haftung in Bezug auf das Dentin etwas erhöhen kann. Grundsätzlich ist $SnCl_2$ als Verbindung mit einem geringen Risiko für Nebenwirkungen klassifiziert und wird in niedriger Konzentration bereits jetzt regelmässig in der Zahnmedizin verwendet. In hohen Konzentrationen und mit saurem pH-Wert sind derartige $SnCl_2$-Lösungen jedoch, ähnlich wie H_3PO_4, potenziell toxisch. Die Toxizität von Phosphorsäure ist bereits umfangreich untersucht worden, dagegen ist über die Toxizität von hochkonzentrierten Zinnchloridlösungen auf zellulärer Ebene wenig bekannt. Ebenso ist unbekannt, inwiefern sich potenzielle toxische Effekte der Einzelkomponenten addieren können. Die Studie hat daher die In-vitro-Zelltoxizität von verschiedenen Verdünnungen von $SnCl_2$ im Vergleich zu H_3PO_4 und dem Primer eines selbstkonditionierenden Adhäsivs (Clearfil SE) sowie von Kombinationen der drei Agenzien in drei verschiedenen Konzentrationen (niedrig, mittel und hoch; 0,01–0,3%) in einem L929-Fibroblasten-Bioassay untersucht.

Material und Methoden

Die Zellvitalität wurde mit einem MTT-(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)-Assay (lichtmikroskopische Analyse und Absorptionsmessung der MTT-Färbung) untersucht, die Integrität der Zellmembranen sowie die Zellmorphologie wurden durch eine Trypan-Blau-Färbung

dargestellt (Zellnekrose-Assay). Alle Experimente wurden zweifach durchgeführt.

Resultate und Diskussion

Die halbmaximale Zellvitalität wurde zwischen einer Konzentration von 0,13% und 0,09% für $SnCl_2$, zwischen 0,09% und 0,06% für H_3PO_4 und zwischen 0,29% und 0,19% für den Clearfil-SE-Primer ermittelt. Die Kombinationen der Einzelagenzien führten zu einer Erhöhung der Toxizität durch additive Effekte, vor allem wenn es sich um eine Kombination mit dem Primer handelte. $SnCl_2$ und H_3PO_4 zeigten unter den vorliegenden In-vitro-Bedingungen ähnliche Zelltoxizität sowie vergleichbare Effekte in Bezug auf die Membranpermeabilität. Diese Ergebnisse zeigen, dass konzentrierte $SnCl_2$-Lösungen grundsätzlich klinisch anwendbar sind. Weitere Studien, etwa an intraoralen Zelllinien, müssen zeigen, ob sich diese Erkenntnisse weiter untermauern lassen. Die vorliegenden Erkenntnisse eröffnen neue Perspektiven für In-situ- und klinische Studien zur Anwendung hochkonzentrierter Zinnpräparate im Rahmen der Adhäsivtechnologie.

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