

Research Article

Antimicrobial Silver-Polyethyleneimine-Polylactic Acid Polymer Composite Film for Coating Methacrylate-Based Denture Surfaces

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To prepare an antimicrobial polymer composite composed of silver- (Ag-) polyethyleneimine- (PEI-) polylactic acid (PLA) in chloroform, for coating the mucosal surfaces of methacrylate-based dentures as a prospective therapy for denture stomatitis. The water-insoluble, tightly bound, hard, micrometre-thin, and colourless film exerts its effects by direct contact with the pathogens and via the active constituents (Ag, PEI, and Ag-PEI) released slowly into the mucosa's salivary layer. Silver and PEI were blended at 140°C, then bound to PLA. The Ag-PEI complex was characterised by dynamic light scattering and transmission electron microscopy, and the Ag-PEI-PLA composite was examined by atomic force microscopy and micro-computed tomography. The characteristic was measured by atomic force microscopy (AFM) and micro-computed tomography (micro-CT). The quantity of water-soluble Ag-PEI complex released from the composite film was measured with gravimetry. The cellular physiological effects were analysed by impedimetry and computer-based morphometry using human gingival epithelial cells. A real-time cell proliferation assay revealed moderate toxic effects of Ag-PEI on the epithelium. The viscous Ag-PEI-PLA solution produced could be applied as a thin film on methacrylate surfaces. Active antimicrobial components (Ag, PEI, and Ag-PEI) were released from the hard, tightly bound Ag-PEI-PLA coating. This study's findings verified the applicability of the antimicrobial Ag-PEI-PLA composite for coating the inner surfaces of acrylate dentures. Owing to the well-known antimicrobial effects of silver and PEI and the supplementary effects of chloroform, this composite provides a new therapeutic method for denture stomatitis that can be easily performed by dentists.

1. Introduction

Denture stomatitis (also known as denture-related stomatitis, candida-associated denture stomatitis, denture-associated erythematous stomatitis, prosthetic stomatitis, or a combination of these terms) is a common, multifactorial condition in which mild inflammation and erythema of the oral mucous membrane occur under a removable prosthesis (i.e., a partial

or complete denture or an orthodontic appliance). In patients having removable prostheses, the prevalence of denture stomatitis ranges from 15% to >70%. The most important etiological factors include ill-fitting dentures, poor dental hygiene, high carbohydrate intake, reduced salivary flow, diabetes mellitus, and immune deficiency. *Candida* species are oral fungal commensal organisms that are present in up to 90% of healthy individuals; *Candida albicans* is the most

frequent fungal coloniser. Although denture stomatitis is not a particularly severe illness in most cases, it may potentiate additional infections in susceptible patients [1–7].

There is a large body of evidence that demonstrates that *Candida albicans* is capable of adhering to polymethylmethacrylate (PMMA) surfaces to form a biofilm. Microbial plaque on the inner surfaces of dentures and the mucosa is composed of bacteria and fungi. Changing the local environment favours dominance of *Candida* and causes mucosal inflammation; bacteria may support these inflammatory reactions. Despite these relationships, most forms of therapy focus on anti-*Candida* treatment [3, 8–10].

The treatment and management of denture stomatitis can be focused locally and/or systemically. For patients with poor compliance, diabetes, or who are immunocompromised, systemic therapy is recommended. In the literature, there are many different forms and local application techniques of antimicrobial agents reported, with very diverse results. The most important issues include the development of resistance and recurrent infections. Currently, there is no “golden rule” for optimal therapy [11–15].

Our goal was to design an antimicrobial, hard polymer composite for coating the inner surface of dentures. This thin, polymer composite film must comply with various requirements: it should be biodegradable, release of the active ingredients from the film must be possible, it needs to be soluble in a suitable solvent, and it must tightly bond to acrylate. Another technical requirement is the easy and rapid preparation of a thin layer of the material by a dentist or the dental laboratory. Although many polymers are used for coating, the most common polymer, polylactic acid (PLA) dissolved in chloroform, was chosen because of the fast and complete elimination from the denture surface by evaporation.

We conceptualised that silver-polyethyleneimine (Ag-PEI), with its core-shell micellar structure, excellent colloidal stability, and easy method of hydrothermal synthesis, would be the optimal active antimicrobial ingredient [16–18]. The synergistic antibacterial effect of silver with polyethyleneimine (PEI) has also been demonstrated [19, 20].

This Ag-PEI nanocomplex is water-soluble; therefore, it is not suitable for acrylate coating on its own. To avoid this disadvantage, we needed to apply a polymer bulk material to form a stable film. We chose PLA, which can easily bind to the Ag-PEI component by electric self-assembly (ESA) and is nearly insoluble in water. The silver-polyethyleneimine-polylactic acid (Ag-PEI-PLA) composite in chloroform is capable of strongly binding to acrylate.

The objective of the present study was to develop an alternative form of therapy that approaches the problem from a new aspect: to kill the pathogens with the Ag-PEI active ingredient, while a suitable coating material simultaneously solubilises the first few superficial micrometres of the acrylate. For this purpose, chloroform promised to be the best solvent in which the Ag-PEI and PLA solubilised equally well. During the coating procedure, the first few superficial micrometres of acrylate will swell and become partially soluble [21], which help kill the pathogens while embedding them.

After the chloroform rapidly evaporates, the composite becomes a thin, hard, and colourless layer. The thickness of

this layer is between 30–40 μm , which does not influence the stability of the prosthesis. Considering the novel characteristics of this form and application of the Ag-PEI-PLA polymer composite, we propose the name “antimicrobial hard lining” or “antimicrobial hard liner” for this form of therapy and the composite.

Although the cytotoxic natures of Ag and PEI to bacteria are well known, we analysed their biophysical effects on the human gingival epithelium (HGEP) using impedimetry. Due to the electrical insulator moiety provided by the phospholipid bilayer of the surface membrane, impedimetry allowed us to analyse some basic cellular physiological responses, including viability, cell adhesion, and cell proliferation, induced by PEI and Ag-PEI.

The present paper discusses the synthesis and characterisation of the Ag-PEI-PLA composite, including its toxicity and adaptability in human therapy.

2. Materials and Methods

2.1. Materials. High-branched polyethyleneimine (PEI) with a molecular weight of 25,000, silver nitrate (AgNO_3), polylactic acid (PLA) with a molecular weight of 60,000, and chloroform were purchased from Sigma-Aldrich Chemie, Germany; they were used in this study without further purification. Deionised and ultrapure water were used throughout the study; their resistivity was $\geq 18 \text{ M}\Omega \text{ cm}$.

2.2. Preparation of the Ag-PEI-PLA Polymer Composite. During the first phase, the Ag-PEI complex, the “antimicrobial active component,” was synthesised using a hydrothermal method: 1 g of PEI was diluted in 2 ml of deionised water by stirring in a glass beaker; in another glass beaker, 158 mg of AgNO_3 (100 mg Ag) was diluted in 1 ml of deionised water by mild shaking the beaker. After the shaking, the solutions were centrifuged for 10 minutes at 20,000g (Micromax RF, Thermo Fisher Scientific, USA). The obtained solutions were slowly unified in the PEI beaker by continuous stirring. The yellowish solution was placed on the plate of a hot plate stirrer (VELP Scientifica, Italy) set at 90°C to evaporate the water. During this process, a continuous darkening of the sample to a deep brown colour and a reduction in its quantity were observed. The temperature was then increased to 140°C; the substance thickened, and the magnetic stir bar ceased moving. Upon completion of this step, the sample was further heated for 4 h, until it became a deep, dark greyish-brown, thick mass. A dissolution test performed on the obtained Ag-PEI sample proved that the prepared substance was water- and chloroform-soluble [22, 23].

During the second phase, the PLA was conjugated to the Ag-PEI complex by ESA. Two millilitres of 5% (w/v) PLA in chloroform was added to 20 mg of the Ag-PEI sample in a glass tube. At this step, the honey-like, viscous Ag-PEI was first smeared onto the wall of the preweighted tube with a spatula, then the mixture was intensely shaken for 30 min in a laboratory shaker (IKA Works, Wilmington, NC, USA). The Ag-PEI was very easily solubilised in the 5% PLA solution, which turned yellowish-brown. Subsequently, a 30 min ultrasonic treatment was performed (45 kHz, 100 W; Emmi

12HC, EMAG, Germany). The practically colourless supernatant was gained with a 2 min, 2000 rpm centrifugation (BW41-BR230, Qualitron Inc., Korea), and the aggregated pellet was discarded. The resultant substance was considered suitable for coating acrylic surfaces [24, 25].

2.3. Atomic Force Microscopy of the Ag-PEI-PLA Composite. The AFM measurements were performed with an atomic force microscope (Dimension Icon, Bruker, Palaiseau, France) in the tapping mode, under ambient conditions (relative humidity, ~50%; temperature, ~22–24°C). The phase images from the tapping mode operation are a map of how the phase of cantilever oscillation is affected by its interaction with the surface. The physical meaning of this signal is complicated, but in addition to topographic information, the phase can be affected by the relative softness/hardness or chemical nature of the sample [26, 27]. For the tapping mode studies, a cantilever (TESPA-V2, Bruker, Palaiseau, France) with a spring constant of approximately 42 N/m and resonance frequencies of approximately 320 kHz was used.

2.4. Micro-Computed Tomography of the Ag-PEI-PLA Composite. The Ag-PEI-PLA polymer composite film was scanned using a micro-computed tomography device (Sky-Scan 1172 microCT, Bruker, Kontich, Belgium). The acquisition parameters were as follows: a 1.91 μm isometric cube voxel with no filter, at 40 kV, with a 200 μA tube current. The rotation step was 0.5°. From the raw image data set, 3D-rendered images were constructed using CTAn and CTVol software (Bruker, Kontich, Belgium).

2.5. Weight Changes of the Ag-PEI-PLA Composite after 8 Days of Distilled Water (DW) Storage. Fourteen-centimetre-squared portions of PMMA microscopic slides (26 \times 76 mm) were coated with 1–1 ml of the Ag-PEI-PLA composite and PLA in chloroform. After evaporation of the chloroform, the dried slides were placed into DW for 8 days. The weight changes of the air-dried slides were measured on days 1, 2, 3, 4, 5, and 8 with an analytic balance.

2.6. Size Measurement of the Nanoparticles Released from the Polyethyleneimine-Polylactic Acid (PEI-PLA) and Ag-PEI-PLA Membranes. Thin polymer membranes were prepared by casting on a glass surface and pulling out carefully. The compositions of PEI-PLA and Ag-PEI-PLA membranes were identical to those described above. Three cut pieces from both types of membranes (area, 1 cm^2 ; thickness, 0.05 mm) were immersed in 5–5 ml cell culture media free of fetal bovine serum (FBS), representing identical extraction times. Samples (1.5 ml) containing the released particles were collected on day 1, day 5, and day 10 (D1, D5, and D10, respectively). The sizes of the nanoparticles released from the PEI-PLA and Ag-PEI-PLA membranes were measured using a Zetasizer Nano S instrument (Malvern Instruments Ltd., Malvern, UK).

2.7. Impedimetry

2.7.1. Model Cells. Cultures of the HGEP cell line (CELLn-TEC, Bern, Swiss) were maintained in CnT-24.S medium

(CELLnTEC, Bern, Swiss) containing 1% L-glutamine and supplemented with 1% penicillin-streptomycin (Lonza Group Ltd., Switzerland).

The cell cytotoxic and adhesion effects of the PEI-PLA and Ag-PEI-PLA extracts were investigated with a xCELLigence RTCA SP (ACEA Biosciences Inc., San Diego, USA) impedance-based system. The real-time assays were implemented in E-plate 96 arrays (ACEA Biosciences Inc., San Diego, USA) with a 15 kHz AC system, using a 20 s sampling frequency on noncoated electrodes.

2.7.2. Cytotoxicity Assay. First, growth of a confluent culture of HGEP cells was controlled by impedimetry in the wells of the E-plate 96 arrays. After 24 h, the cultures were treated with the 1- or 5-day PEI-PLA or Ag-PEI-PLA membrane extracts (dilutions: 1000x, 100x, 10x, and 1x) for 168 h.

2.7.3. Cell Adhesion Assay. First, the impedance of the culture medium was recorded as a baseline and absolute control. Second, the HGEP cells were loaded together with extracts of PEI-PLA or Ag-PEI-PLA (dilutions: 1000x, 100x, 10x, and 1x) prepared by soaking the composite membrane in cell culture medium for 1 or 5 days. The follow-up time was 12 h.

2.8. Apoptosis Measurement. In the background of the cytotoxic effects, induction of apoptotic processes is frequently revealed. In the present study, our objective was to detect early apoptotic events. The ratio of apoptotic HGEP cells was evaluated after 24 h of treatment with the PEI-PLA and Ag-PEI-PLA composite extracts. For this purpose, two independent apoptosis assays were performed: (i) computer-based morphometry analysis of HGEP cells treated with the composite extracts and (ii) annexin V staining which is a well-known indicator of the disturbed surface membrane asymmetry, a chief early marker of apoptosis. Computer-based evaluation of the dead cell number on micrograph series taken by light microscopy (obj.: 20x; Axio Observer A1; Carl Zeiss Microscopy GmbH, Jena, Germany) was done by Fiji ImageJ software [28]. The early phase of apoptosis was detected using annexin V labelling (PE Annexin V, BioLegend, San Diego, USA) and was measured by flow cytometry (FACSCalibur, BD Biosciences, NJ, USA).

2.9. Statistical Analysis. The delta CI (ΔCI) and slope values were calculated by the integrated software (RTCA 1.2, Roche Applied Science, Indianapolis, IN, USA) of xCELLigence SP System. For the further analysis of the data, the Origin Pro 8.0 (OriginLab Corporation, Northampton, MA, USA) statistical program was used. Data shown in the figures represent mathematical averages of three parallels and $\pm\text{SD}$ values. Statistical analysis of data was done by the application of ANOVA of Origin Pro 8.0. The level of significance is shown as follows: $x: P < 0.05$; $y: P < 0.01$; $z: P < 0.001$.

3. Result

3.1. Weight Changes of the Composite after 8 Days of DW Storage. Initially, the average weights of the Ag-PEI-PLA and the control PLA layers prepared on the microscopic glass slides were 57.58 mg and 58.76 mg, respectively. After 8 days,

the weights of both the composite and PLA film had decreased, but the weight lost from the composite was significantly higher than that of the PLA film (33% and 11%, respectively, on day 8; Figure 1).

3.2. Size of the Nanoparticles Released from the PEI-PLA and Ag-PEI-PLA Membranes. Figure 2 presents the size distribution analyses of the particles released from the PEI-PLA and Ag-PEI-PLA membranes and demonstrates that they have characteristic profiles containing populations of particles significantly different from that of the control (medium). A time-course analysis of the peaks shows a decrease with time (Ag-PEI-PLA: D1, 7 nm; D5, 6 nm; D10, 4 nm); nevertheless, no significant difference was detectable in the PEI- vs. Ag-PEI-containing preparations in relation to the duration of the study (data not shown).

3.3. Atomic Force Microscopy of the Ag-PEI-PLA Composite. The pseudo-3D images of the height data show spherical shapes in the range of 0.5–4.0 μm (Figures 3(b) and 3(d)). In this case, the phase images were primarily determined by the topographic structure on the thin film surface. The phase images provided better contrast than the height images (Figures 3(a) and 3(c)).

3.4. Micro-Computed Tomography of the Ag-PEI-PLA Composite. At the maximum enlargement provided by our micro-CT instrument, a granular structure of the composite was evident, indicating the aggregation process (Figure 4).

3.5. Impedimetry

3.5.1. Cytotoxicity. The toxic effects elicited by the nanoparticles released from the PEI-PLA and Ag-PEI-PLA were evaluated in the day 1 and day 5 samples. The effects of the 1/10 serial dilutions in the range of 1000x–1x were analysed by impedimetry over a 0–168 h period. As depicted in Figure 5, the day 1 samples were capable of eliciting strong and durable cytotoxic effects in the HGEP cells. The cytotoxic sensibility of the HGEP cells proved to be greater to the PEI-PLA D1 extracts (dilution range, 100x–1x) than the Ag-PEI-PLA extracts (dilution range, 10x–1x). For the day 5 extracts (data not shown), the cytotoxic characters of both types of extracts were still detectable; however, the amplitude of toxicity in the PEI-PLA extract was similar to that of the Ag-PEI-PLA extract.

3.5.2. Cell Adhesion. Comparison of the adhesion blocker abilities (Figure 5) demonstrated that both the PEI-PLA and Ag-PEI-PLA day 1 extracts had a significant blocking effect (dilution ranges: 1x–10x); however, the effect of the PEI-PLA extract was more pronounced than that of the Ag-PEI-PLA extract.

3.6. Apoptosis. Our data revealed that both the day 1 and day 5 PEI-PLA and Ag-PEI-PLA extract samples resulted an increased number of dead cells (Table 1) as well as annexin V signals (Figure 6). A comparison of the apoptotic effects elicited by the nanoparticles derived from the PEI-PLA and Ag-PEI-PLA extracts indicated that PEI alone had the

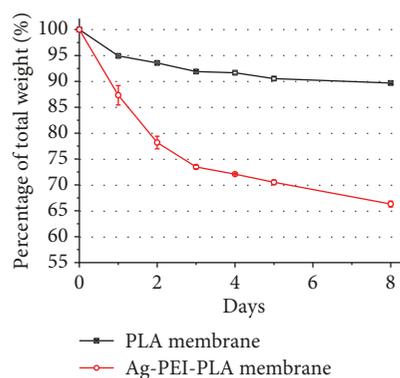


FIGURE 1: Weight changes of the polymer composite (silver-polyethyleneimine-polylactic acid [Ag-PEI-PLA]) and the poly(lactic acid) (PLA) coating in distilled water (DW) over an 8-day period. Technical parameters of the assay—sample number: 16; temperature: 25°C; time: 8 days. The error bars represent the standard deviation of measurements for 8–8 samples in 8 days ($n = 96$).

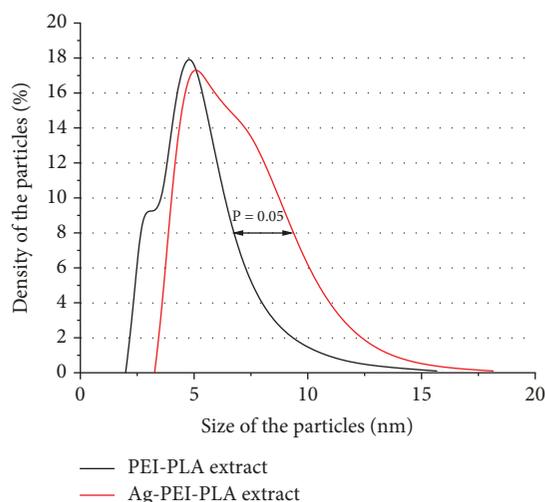


FIGURE 2: Size distribution of the day 1 polyethyleneimine-poly(lactic acid) (PEI-PLA) and silver-polyethyleneimine-poly(lactic acid) (Ag-PEI-PLA) extracts (medians: PEI-PLA extract, 4.8; Ag-PEI-PLA extract, 5.6). The detected difference indicates that the size of the silver-containing PEI-PLA particles was significantly larger ($P = 0.05$) than that of the PEI-PLA particles. Technical parameters of the assay—sample number: 3; temperature: 25°C; time: 60 s.

strongest and widest range (100x–10x) effect, while in Ag-PEI, only 10x dilutions were effective.

4. Discussion

Our goal was to extend the possibilities for the management and therapy of denture stomatitis beyond conventional methods. The aim of this study was to produce a thin, hard film on the mucosal surface of acrylate dentures that has antimicrobial effects. Beyond the advantageous on contact killing property of this hard antimicrobial surface, this film has

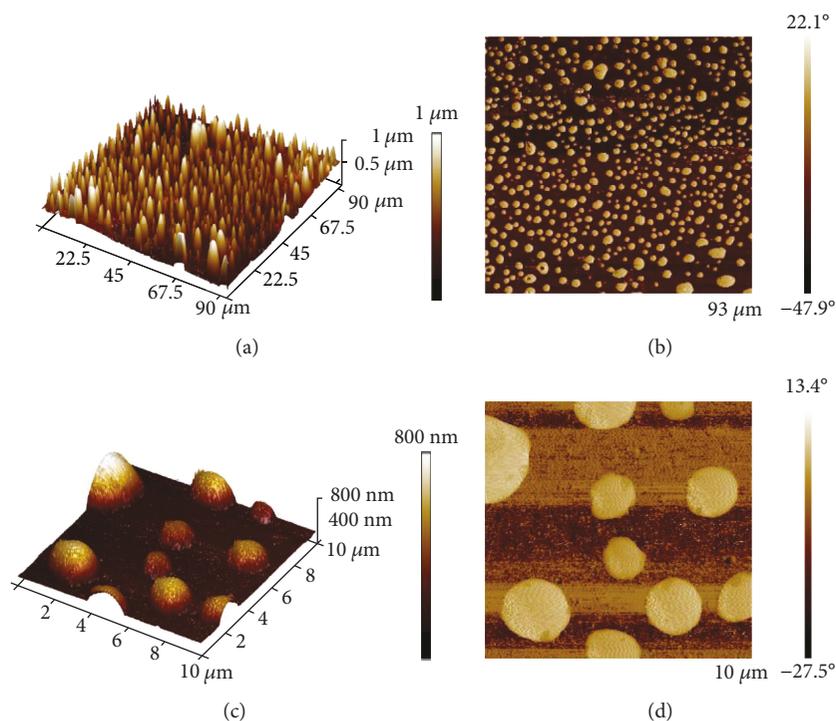


FIGURE 3: Representative atomic force microscopy (AFM) images of the silver-polyethyleneimine-poly(lactic acid) (Ag-PEI-PLA) polymer composite obtained in the tapping mode. (a) A 3D AFM height topography large area scan of the Ag-PEI-PLA complex. (b) A 2D AFM phase large area scan of the Ag-PEI-PLA complex. (c) An enlarged view of the 3D AFM height topography scan of the Ag-PEI-PLA complex. (d) An enlarged view of the 2D AFM phase scan of the Ag-PEI-PLA complex. Relative humidity, $\sim 50\%$; temperature, $\sim 22\text{--}24^\circ\text{C}$.

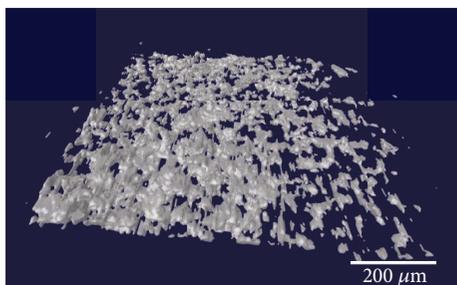


FIGURE 4: A micro-computed tomography image of the Ag-PEI-PLA composite. Technical parameters of the assay— $1.91\ \mu\text{m}$ isometric cube voxel with no filter, at 40 kV, with a $200\ \mu\text{A}$ tube current.

additional effects owing to the release of active components. For easy application of the polymer composite on the inner surfaces of prostheses, it is necessary for it to have a suitable viscosity and rapid evaporation of the solvent and be insoluble or very slow to solubilise in water.

The presented work is focused on basic research, dealing with *in vitro* assays; nevertheless, authors complement it with some recommendations for the clinical usage. The application of this hard antimicrobial liner is very similar to the application of the adhesive layer used in the classical relining procedure of dentures by brush. After the evaporation, a longer storage in water can induce the release of the active complex.

The silver nanoparticles, which have some unique properties, play a central role in antibacterial and antifungal therapies [29]. The effectiveness of these nanoparticles depends on the performance of adequate laboratory procedures during preparation [29–33]. There are more than one hundred reports regarding the combination of silver with different stabilisers, including polymers like polyethyleneimine, while avoiding strong aggregation [17, 18, 19, 34, 35]. All the authors used several alterations, but the basic steps of synthesis include the reduction of silver and inhibition of aggregation, followed by functionalization for a given role. The antimicrobial effectiveness primarily depends on the size of the nanoparticles [36, 37].

The method used in the present study uses an easy and common form of synthesis, with mild reduction and aggregation inhibition by PEI. The dense Ag-PEI complex is suitable for long-term storage, and a new sample can be easily prepared at chairside for application. According to generally accepted professional opinion, the smaller silver clusters or aggregates provide higher antimicrobial effects [36, 37]. In our composite, the PEI was used for three purposes: (1) the cationic polymer's antimicrobial effect, (2) the colloidal stability of nanosilver, and (3) the reduction of silver ions in the AgNO_3 solution. The antimicrobial effects of Ag and PEI are well known, both individually and in combinations [20, 38–40].

For coating purposes, instead of the denture material (PMMA) which is nonbiodegradable, PLA was used in compliance with the previously stated requirements: it is

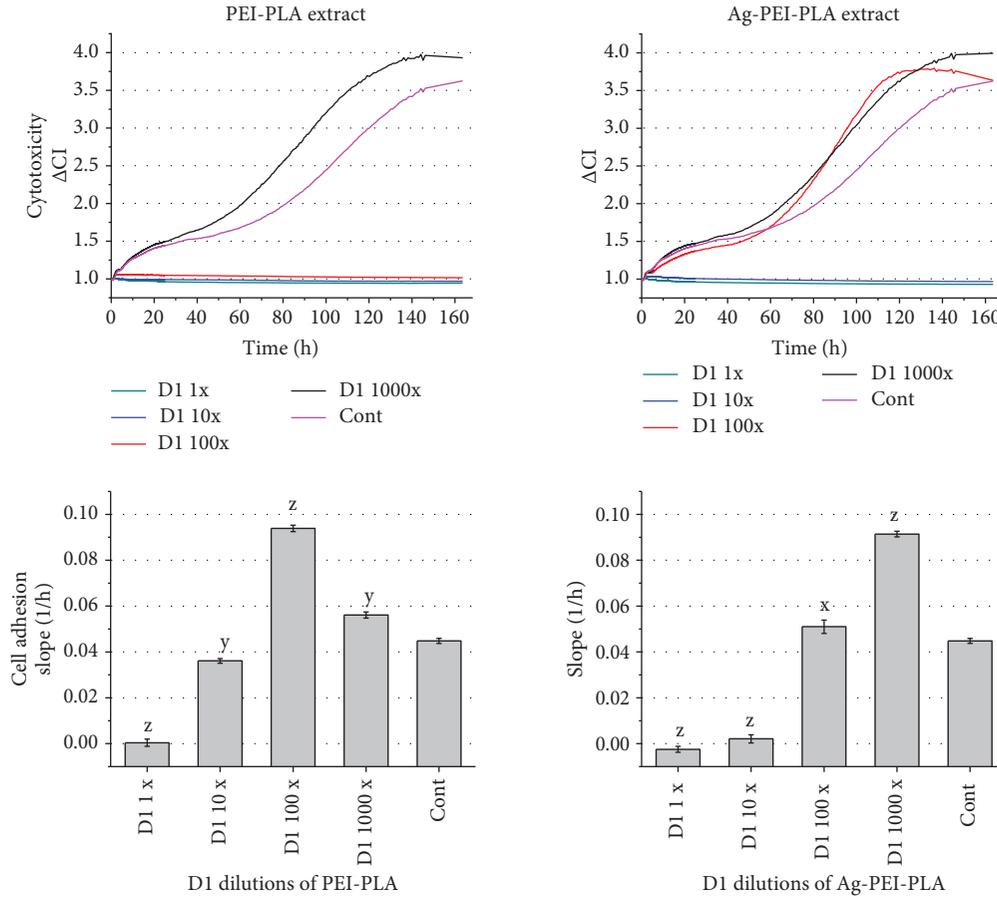


FIGURE 5: Effects of the polyethyleneimine-polylactic acid (PEI-PLA) and silver-polyethyleneimine-polylactic acid (Ag-PEI-PLA) extracts (day 1 [D1] extraction: dilution range, 1000x–1x) on the cytotoxicity and cell adhesion of the human gingival epithelium cells using impedimetry (x : $P < 0.05$; y : $P < 0.01$; z : $P < 0.001$). Delta cell index (ΔCI); control (Cont). Technical parameters of the assay—sample number: 5/measuring point; temperature: 37°C; time: 160 h.

TABLE 1: Computer-assisted morphometry of apoptosis induced by polyethyleneimine-polylactic acid (PEI-PLA) and silver-polyethyleneimine-polylactic acid (Ag-PEI-PLA) extracts. D1: day 1; D5: day 5; and D10: day 10 extractions. Serial dilutions of 1/10, range: 1000x–10x. Control (Cont) in HGEp cells.

	Number of apoptotic cells [\pm SD] PEI-PLA	P	Apoptosis	Number of apoptotic cells [\pm SD] Ag-PEI-PLA	P	Apoptosis
Control	1 \pm 0.32			1 \pm 0.54		
D1–10x	103 \pm 3.21	0.001	+++	20 \pm 2.33	0.01	+
D1–100x	37 \pm 2.14	0.01	+	1 \pm 0.24		
D1–1000x	3 \pm 0.44			1 \pm 0.31		
D5–10x	86 \pm 3.03	0.001	++	24 \pm 3.67	0.01	+
D5–100x	48 \pm 3.61	0.01	+	2 \pm 0.78		
D5–1000x	3 \pm 0.38			1 \pm 0.12		
D10–10x	70 \pm 4.70	0.01	++	45 \pm 4.11	0.01	+
D10–100x	35 \pm 3.63	0.01	+	4 \pm 0.41		
D10–1000x	2 \pm 0.15			1 \pm 0.09		

biodegradable, soluble in chloroform, and easily binds to the Ag-PEI nanoparticles by ESA. Several methods are available for preparation of the PEI-PLA copolymer, and different polymer combinations are available for drug delivery systems [41–44].

Additionally, the solubilising agent, chloroform, has an antipathogenic effect. It is one of the strongest permeabilising organic solvents with antipathogenic effects on *Candida albicans* [45, 46], and it facilitates intake of active components (e.g., Ag and PEI) into the bacterial and fungal cells. Another

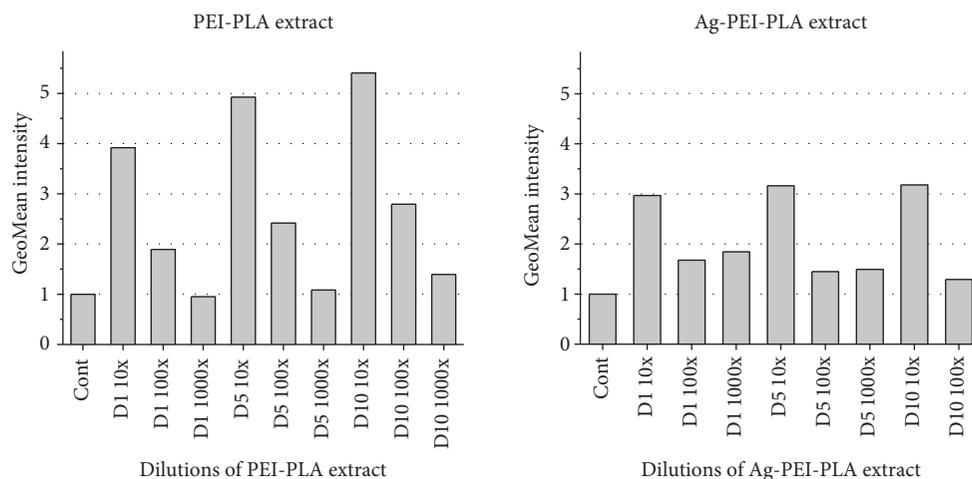


FIGURE 6: Apoptotic potencies of the polyethyleneimine-poly(lactic acid) (PEI-PLA) and silver-polyethyleneimine-poly(lactic acid) (Ag-PEI-PLA) extracts. D1: day 1; D5: day 5; and D10: day 10 extractions. Serial dilutions of 1/10, range: 1000x–10x. Control (Cont).

important property of chloroform is that it is a weak hydrogen donor, which may influence the ESA and the release of components [47, 48].

The Ag^+ mild and PEI have medium cytotoxic effects, respectively. All antimicrobial agents have some degree of cytotoxic effect. The degree of cytotoxicity depends on the sensitivity of the microbial species, the characteristics of the environment, and in our study, the modification of agents, notably PEI. The cytotoxicity of PEI depends on the number of reactive amino groups at the free terminals of the chains [49]. Forming complexes with Ag^+ , the number of free PEI amino groups will decrease. Our comparative evaluation of the extracts revealed that the Ag-PEI-PLA extract had significantly less cytotoxic effects than the derivative PEI-PLA extract containing no silver. The time course evaluation of nanoparticle release (day 1, day 5, and day 10) also demonstrated that over a long period, the Ag-PEI-PLA extract is more advantageous, as it has less apoptosis induction ability. To summarise, the cell biology analysis data show that the nanosilver-containing novel composite (Ag-PEI-PLA) is better tolerated by the cells of the superficial layers of the oral cavity.

One of the most important clinical requirements of this study was to achieve an antimicrobial effect that extended beyond a week. For practical application of our composite, we need to know when the coating layer partially or completely disappears from the PMMA surface. In a pilot experiment, we measured the weight changes of the composite- and PLA-coatings alone as controls after storage in DW. Theoretically, the water-soluble Ag-PEI active component should release first. The micellar structure and nanospaces between its individual elements allow the possibility of a slow release of different components via diffusion. This phenomenon may also assist PLA degradation, which is a very slow process. In the literature, there are no data reported regarding the interaction between the individual parts of the composite, or in our case, between PLA and PEI on release.

Over the antibacterial contact killing, the effect of the released components is also notable. As demonstrated in

Figure 1, the weight of our composite decreased over the 1–8-day period was 33.69%. This result was significantly higher than the PLA release. According to our concept, the PLA, through ESA, decreased the solubility of the PEI or Ag-PEI. The ratio of the PLA and active components will influence the rate of release. These data indicate that the composite's weight loss was greater than the entire Ag-PEI content (mean, 16.67%) in the composite sample. It is possible that the released substance was a mix of the components. From another perspective, the weak hydrogen bonding of the solvent (chloroform) may influence the physical properties of the polymers and decrease the molecular mobility [47, 48].

The antimicrobial effects of silver and PEI are well known, but the exact, detailed analysis of the Ag-PEI-PLA composite on the different pathogens of oral cavity is necessary to be studied. Investigation of the composite is planned to be extended in microbiology and clinical practice.

Further research is required to investigate the viability of the pathogens in the deeper layers of the microcracks covered by the microbial film and to determine the potential effects caused by the modification of surface roughness.

5. Conclusions

In this study, a new method was presented for consideration as a novel antimicrobial surface-modifying substance for managing denture stomatitis. The essence of the method was to prepare a nanosilver-PEI-PLA substance, in chloroform, for coating the mucosal surface of removable dental prostheses. The antimicrobial effects are exerted primarily by Ag and PEI. Additionally, the chloroform had a solubilising effect on the thin, upper acrylate layer of the prosthesis, which may enhance the antimicrobial effects by permeabilising the pathogen membranes. The authors are convinced that this new method, including its multiple combined antimicrobial effects, provides an opportunity to introduce an effective, novel candidate for managing denture stomatitis. Further, this application procedure could be easily performed chair-side by dentists.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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