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Two-component periodontal depot for sustained release of Chlorhexidine

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Abstract: Periodontitis is a prevalent chronic inflammatory disease that requires effective long-term treatment strategies. Current antimicrobial formulations, such as Chlorhexidine (CHX) gels and antibiotic-based inserts, often suffer from short retention times and uncontrolled drug release, limiting their therapeutic efficacy.

To address these challenges, we developed a biodegradable, *in situ* cross-linked CHX depot incorporating a bifunctional reactive cross-linker, triethylene glycol bis(dilactic acid) bis(hexamethylene diisocyanate) (TELA-NCO). The synthesized TELA-NCO was mixed with commercially available CHX gel to form an injectable paste that polymerizes within 7 hours into a stable depot. HPLC release studies demonstrated sustained CHX release for over 35 days, significantly reducing initial burst effects. *In vitro* cytocompatibility tests revealed expected concentration-dependent CHX cytotoxicity, but good biocompatibility of the cross-linker. This injectable, degradable depot system offers a promising alternative for localized antimicrobial therapy in periodontology and endodontics, eliminating the need for secondary removal.

Keywords: Periodontal Application, Drug Depot, Chlorhexidine

1 Introduction

Periodontitis is one of the most prevalent chronic inflammatory diseases, affecting millions worldwide [1]. Due to demographic changes, an increased need for effective treatment strategies is anticipated in the future. These chronic inflammatory processes interact with other general medical conditions [2]. Pronounced periodontitis represents an enormous physical burden, comparable to an open wound the size of the palm of the hand. As patients with periodontitis have a proven higher risk of cardiovascular disease (e.g. myocardial infarction, apoplexy), the far-reaching clinical significance of periodontitis should be emphasized in this context. In addition to diseases of the marginal periodontium, bacteria also trigger inflammatory processes in the jawbone from necrotic root canals.

Modified medicated inserts play an important role in improving the prognosis of the treated teeth. For the treatment of periodontitis, peri-implantitis, and endodontics, various active substances and drug delivery systems are available, differing in their mode of action. However, current approaches often face challenges such as limited retention time and suboptimal drug release profiles, which may reduce therapeutic effectiveness. Chlorhexidine is widely used as an anti-infective compound, available in rinses, gels, and gelatin chips (e.g. Periochip), as well as a xanthan-based gel (e.g. ChloSite) [3]. Additionally, doxycycline-based preparations, such as Ligosan, represent locally applied antibiotic options [3]. Despite the availability of various CHX-based formulations, their effectiveness is often compromised by short retention times and uncontrolled drug release, necessitating frequent reapplications. This is particularly challenging in the context of often low patient adherence.

To overcome these limitations, we propose a biodegradable CHX depot that ensures prolonged retention and controlled drug release through *in situ* polymerization. This system integrates an advanced bifunctional reactive cross-linker [4, 5] based on isocyanate-functionalized tri-ethylene glycol bis(dilactic acid) (TELA-NCO), ensuring prolonged retention at the treatment site, and a customizable active ingredient component for sustained and controlled drug release. The use of such an active ingredient depot in addition to existing, commercially available pasty active ingredient formulations enables the release of patient-specific, customized active ingredients. The cross-linker increases the retention time and enables a better controlled release of the active ingredient quantity over time.

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2 Materials and Methods

2.1.1 Synthesis of TELA-NCO

L-lactide, triethylene glycol and hexamethylene diisocyanate (all: Sigma Aldrich/ Merck KGaA, Darmstadt, Germany) were of technical grade and used as received. CHX gel (Chlorhexamed 1%) was purchased from GalaxoSmithKline Consumer Healthcare GmbH & Co. KG, Munich, Germany.

TELA was synthesized via acidic ring-opening polymerization of L-lactide with triethylene glycol at 130°C (H_3PO_4, 0.1%) under exclusion of humidity, followed by reaction with hexamethylene diisocyanate (HMDI) under Argon at 55°C to obtain TELA-NCO (Figure 1). The final product was stored under Argon until further use.

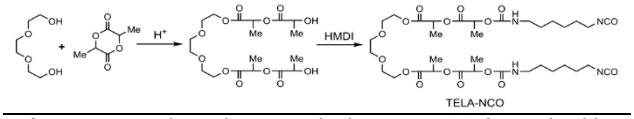

Figure 1: Reaction scheme to obtain TELA-NCO from L-lactide, triethylene glycol and hexamethylene diisocyanate (HMDI) in a two-step reaction.

Depots consisting of 1:1 v/v TELA-NCO/CHX gel were prepared by centrifuge mixing (Hauschild, Hamm, Germany).

2.1.2 ATR-FTIR spectroscopy

Fourier transform infrared spectroscopy - attenuated total reflectance (FTIR-ATR) measurements were performed using a Bruker Vertex 70 IR-Spectrometer (Bruker Optics, Leipzig, Germany) equipped with a DLaTGS-detector. Data were collected in the range of $\tilde{\nu}$ = 700 cm^{-1} to 4000 cm^{-1} with a resolution of $\tilde{\nu}$ = 4 cm^{-1} averaged over 32 scans in reflection mode using a Graseby Golden Gate Diamond ATR-unit. All spectra were subsequently baseline corrected and atmospheric compensation was performed by Bruker's OPUS software.

2.1.3 CHX release experiments

Depots consisting of 1:1 v/v TELA-NCO and CHX gel were polymerized overnight in screw-cap glass vials. Subsequently, release studies of the Chlorhexidine D-gluconate from the depot were carried out in isot. NaCl at 37 °C with a medium volume of 10 mL per 100 mg Depot.

High Performance Liquid Chromatography (HPLC, Knauer, Berlin, Germany) was used to quantify CHX release as described before [6], using an Eurospher 100 C18 250 x 4 mm column, mobile phase: acetonitrile/0.08M NaH_2PO_4 + 0.5% triethylamine, pH = 3.0 (350/650 v/v), isocratic flow rate: 1 ml/min, temperature: 20 °C, detection wavelength: UV 251 nm, injected sample volume: 20 µl. The calibration was performed in a concentration range from 0.2 to 50 mg/L. Acetonitrile (ACN) and water were HPLC-grade and purchased from Carl Roth, Karlsruhe, Germany. Formic acid was purchased from Fisher Chemicals, Schwerte, Germany.

2.1.4 *In vitro* cytocompatibility

EA.hy926 and HT1080 cell lines were purchased from ATCC, Manassas, VA, USA. MEM and DMEM were purchased from PAN-Biotech GmbH, Aidenbach, Germany.

In vitro cytocompatibility was investigated following the ISO 10993-5 eluate testing protocol [7]. In brief, ~1g of each freshly mixed TELA-NCO/CHX depot, pure TELA-NCO, and pure CHX gel were given to a glass vial and directly covered with cell culture medium at a sample-to-medium ratio of 1 g per 5 mL (MEM for EA.hy926, DMEM for HT1080, each supplemented with 10% FCS and 1% penicillin/streptomycin). Samples were incubated in the medium at 37 °C for 24 h, during which time TELA-NCO/CHX mixture and pure TELA-NCO cured in the medium. After incubation, 10,000 cells per 96-well were pre-cultured over 24 h and subsequently incubated for 48 h with 200 µL of the extracts in decreasing concentrations (100%, 50%, 25%, 12.5%, 6.25% and 3.125%) in 4 replicates under standard *in vitro* conditions (37 °C, 5% CO_2, 21% O_2) in an incubator (ICO240med, Memmert GmbH, Schwabach, Germany). Tissue culture polystyrene (TCPS) and high density polyethylene (HDPE) served as a control. Cell metabolic activity was assessed using CellQuanti Blue Assay (BioAssay Systems, Hayward, CA, USA).

3 Results and Discussion

TELA-NCO is a chemical modification of a previously reported degradable cross-linker [4, 5], offering improved flow properties due to the incorporation of a mobile triethylene glycol unit. This enhances its injectability, allowing the formation of *in situ* matrices that conform to the application site. Upon reaction with alcohol-containing compounds, such as the hydroxypropyl cellulose in CHX gel, a polyurethane network is formed, ensuring curing of the depot *in situ* and localized sustained drug release. Additionally, the lactic acid backbone enables controlled biodegradation.

In this study, TELA-NCO was mixeed with commercially available CHX gel in the ration 1:1 v/v, yielding an easily injectable paste suitable for application via standard 1 mL syringes (Figure 2).

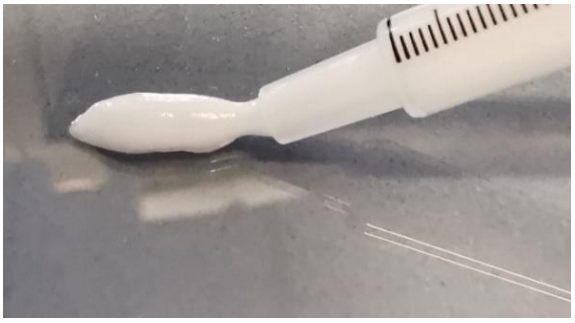

Figure 2: Two component periodontal depot consisting of TELA-NCO and CHX gel after mixing, applied by a standard 1 mL syringe.

The chemical curing of the two-component system was monitored using FTIR-ATR spectroscopy. For this purpose, the premixed paste was applied directly to the measuring crystal and one spectrum per hour was recorded over a period of 48 hours (Figure 3). After 7 hours, during which no further changes in the spectra occurred, the reaction was considered complete and the depot system was regarded as cured.

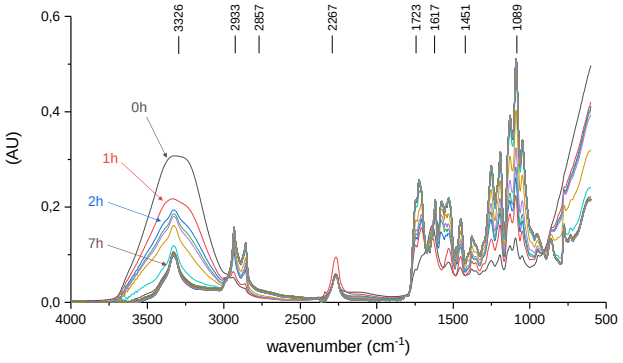

Figure 3: Representation of the time-resolved FTIR-ATR spectra of the curing process of the two-component periodontal depot. The relevant bands and spectra after 0 h, 1 h, 2 h and 7 h are labelled.

The biocompatibility of the two-component depot system was examined *in vitro* on endothelial cells (EA.hy926) and fibroblasts (HT1080) (Figure 4). The experimental cross-linker exhibited good biocompatibility whereas the pure CHX gel was not biocompatible in all dilutions tested. The cytotoxic effect showed a concentration dependency in all depots, so that the negative effect on the cells in the culture can be attributed exclusively to the effect of CHX. The depot system would therefore be suitable in principle for dental application

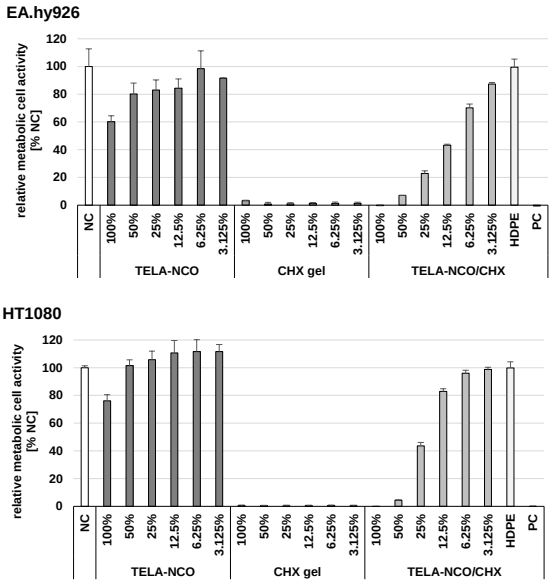

Figure 4: *In vitro* cytocompatibility of TELA-NCO, CHX gel and the mixture of TELA-NCO/CHX gel with endothelial cells (EA.hy926) and fibroblasts (HT1080) with 4 replicates each.

Release studies of the Chlorhexidine from the depot were performed in isot. NaCl at 37 °C. The amount of CHX released was determined using the HPCL method described. CHX is highly soluble in water and a high level of release occurs at the beginning of the release study (Figure 5 A).

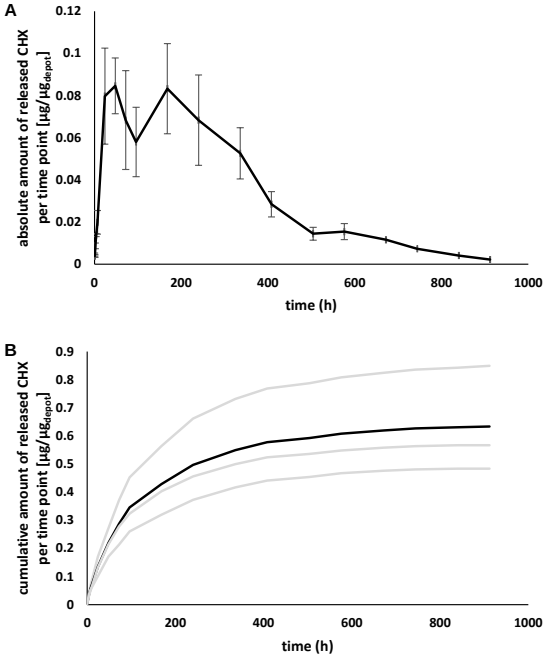

Figure 5: Absolute released amount of CHX per time-point (A) and cumulative released amount of CHX (B) over a period of 38 days (912 h). Black line is mean values over all measurements, grey lines represent single sample values.

The depot demonstrated a sustained CHX release for over 35 days (840 h), in contrast to the immediate CHX release of Chlorhexamed gel. The polyurethane network formed by TELA-NCO cross-linking presumably contributes to slower diffusion kinetics, enhancing retention at the application site (Figure 5 B), in contrast to an application of pure CHX gel, which is associated with an immediate release of the active substance without a depot effect. The variations within the samples with regard to the absolute amount of CHX released are presumably due to inhomogeneities within the depot bodies caused by incomplete mixing and due to the different densities of the two components during volume- based sampling.

4 Conclusion

The presented TELA-NCO/CHX depot provides a promising alternative to conventional formulations by enabling controlled, long-term CHX release in periodontal therapy. Its injectability, degradability, and ease of application position it as a viable candidate for clinical translation. However, further *in vitro* and *in vivo* studies have to be conducted to optimize degradation kinetics with respect to the harsh dental environment and to confirm long-term biocompatibility.

Author Statement

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