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ORIGINAL ARTICLE

Guaiacol/β-cyclodextrin for rapid healing of dry socket: antibacterial activity, cytotoxicity, and bone repair—an animal study

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Abstract

Purpose Dry socket (DS) is one the most common and symptomatic post-extraction complications; however, no consensus on its treatment has been reached. This study aimed to develop a novel dressing material for DS containing the phenolic agent guaiacol and evaluate its biological properties.

Methods An inclusion complex of guaiacol and β-cyclodextrin (Gu/βcd) was prepared by freeze-drying. Its antibacterial activity over six oral bacteria was analyzed using the microdilution method, and its cytotoxicity in osteoblasts was assessed with the MTT assay. The alveolar healing process induced by Gu/βcd was evaluated histologically after the treatment of DS in rats.

Results βcd complexation potentiated Gu's antibacterial effect and reduced its cytotoxicity in osteoblasts. Bone trabeculae were formed in the alveolar apices of rats treated with Gu/βcd by day 7. On day 14, woven bone occupied the apical and middle thirds of the sockets; on day 21, the entire alveolus was filled by newly formed bone, which was in a more advanced stage of repair than the positive control (Alvogyl™).

Conclusion The improvement in Gu's biological properties in vitro and the rapid alveolar repair in comparison with Alvogyl™ in vivo demonstrated the benefits of the Gu/βcd complex as a future alternative for the treatment of DS.

Keywords Antibacterial agent · Cytotoxicity · Dry socket · Guaiacol · Oral surgery · Animal study

Introduction

Tooth extraction is a routine procedure in dentistry, and dry socket is among the most common and symptomatic post-extraction complications [1, 2]. Despite its high incidence, no consensus on the treatment of dry socket has been reached [3, 4], and some proposed protocols have been based on weak scientific evidence [4, 5].

As other phenolics, guaiacol (Gu) has analgesic [6], antioxidant [7], and antimicrobial [8, 9] properties. Gu also stimulates osteogenesis-related genes [6] and is less cytotoxic than other well-known phenolics, such as eugenol [10, 11]; thus, its use as an alternative treatment for dry socket should be explored. Despite its beneficial properties, Gu is difficult to manipulate and store due to its low stability and high volatility [12, 13]. To improve its properties and increase its clinical applicability, we proposed the formation of an inclusion complex of Gu with β-cyclodextrin (βcd).

Cyclodextrins are cyclic oligosaccharides used to moderate drugs' physicochemical characteristics, increasing bioavailability and biological activity [14, 15]. Previous studies involving Gu/βcd inclusion complex development have focused on its physicochemical characterization [16, 17], and not on its possible clinical uses. To the best of our knowledge, it has never been used in dentistry.

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This study aimed to prepare a Gu/βcd inclusion complex, evaluate its cytotoxicity and antibacterial activity, and observe the healing process in rat alveoli treated with Gu/βcd, towards a future clinical application in the treatment of dry socket.

Materials and methods

Materials

Table 1 presents the materials that were used in each experimental phase. All of the solvents used were analytical grade. *Escherichia coli* (*E.coli*), *Staphylococcus aureus* (*S. aureus*), *Streptococcus mitis* (*S. mitis*), *Streptococcus mutans* (*S. mutans*), *Streptococcus sanguis* (*S. sanguis*), and *Aggregatibacter actinomycetemcomitans* (*A.a*) were obtained from the American Type Culture Collection (ATCC).

Inclusion complex preparation

The Gu/βcd inclusion complex was prepared at a 1:1 M ratio using the freeze-drying method [18]. Briefly, βcd was dissolved in Milli-Q™ water (Millipore Corporation, Billerica, MA, USA) at room temperature. Gu was then added, and the solution was stirred for 24 h at 25 °C. The solution was lyophilized for 5 days, until a powder was obtained.

Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy was employed to prove the formation of an inclusion complex. NMR is a powerful tool for the examination of drug–cyclodextrin interactions because it enables identification of host–guest complex interaction sites. Short distances between the atoms of both molecules indicate that they interact in solution to form a real inclusion complex, with different physicochemical characteristics, and not just a physical mixture. 2D ^{1}H-^{1}H ROESY (rotating-frame overhauser effect spectroscopy) spectral analysis was performed using an AVANCE Brucker DRX-400 spectrometer (Bruker Corporation, Billerica, MA, USA) at 400 MHz and 300 K and recorded using the inversion sequence 90-t-180. DMSO-d_6 (99.5% isotopic purity) was used as the solvent. Tetramethylsilane was used as the internal standard (δ 0.0). The ^{1}H NMR spectra of Gu and βcd were assigned according to Song et al. [17] and Schneider et al. [19], respectively.

Table 1 List of materials

Material (abbreviation)	Company (place of origin)	Usage
β-Cyclodextrin (βcd)	Mchem Farma Group Ltd. (Xiamen, China)	PC, CV, A, H
Guaiacol (Gu)	Sigma-Aldrich Co. (St. Louis, USA)	PC, CV, A, H
Dulbecco's modified Eagle's medium (DMEM)	Sigma-Aldrich Co. (St. Louis, USA)	CV
Roswell Park Memorial Institute culture medium (RPMI)	Sigma-Aldrich Co. (St. Louis, USA)	CV
Hemin	Sigma-Aldrich Co. (St. Louis, USA)	A
DMSO-d6	Sigma-Aldrich Co. (St. Louis, USA)	PC
Menadione	Sigma-Aldrich Co. (St. Louis, USA)	A
Trypsin-EDTA	Sigma-Aldrich Co. (St. Louis, USA)	CV
Paraformaldehyde	Sigma-Aldrich Co. (St. Louis, USA)	H
Fetal bovine serum (FBS)	Gibco-Invitrogen Co. (New York, USA)	CV
Penicillin–streptomycin	Gibco-Invitrogen Co. (New York, USA)	CV
Collagenase type II	Gibco-Invitrogen Co. (New York, USA)	CV
3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT)	Gibco-Invitrogen Co. (New York, USA)	CV
Sodium dodecyl sulfate (SDS)	Biotecnologia (Cotia, Brazil)	CV
Brain heart infusion broth (BHI)	Himedia (Mumbai, India)	A
Mueller Hinton broth	Difco (Detroit, USA)	A
Ethylenediaminetetraacetic acid (EDTA)	Synth (Diadema, Brazil)	H
Xylazine	Agribrands Brasil Ltda (Paulínia, Brazil)	H
Ketamine chloride	Agribrands Brasil Ltda (Paulínia, Brazil)	H
Epinephrine	Ariston Ltda (São Paulo, Brazil)	H
Acetaminophen	Germed Pharma (Vinhedo, Brazil)	H
Alvogyl™	Septodont (Saint-Maur-des-Fossés Cedex, France)	H

Study phases: preparation and characterization (PC), cell viability (CV), antimicrobial activity (A), histological observation (H)

Cell viability

Calvaria-derived osteoblasts were isolated from six 5-day-old Wistar rats at the Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. The institutional Ethics Committee guidelines for the care and use of laboratory animals have been observed (approval no. 109/2014). The calvaria was removed and treated enzymatically with 0.05% trypsin-EDTA (5 min), followed by four sequential incubations with collagenase type II solution (1.6 mg/mL) in phosphate-buffered saline (20 min each). The supernatant was then collected and centrifuged (1400 rpm for 10 min), and the pellet was resuspended in RPMI culture medium supplemented with FBS (10%) and penicillin–streptomycin (100 IU mL^{-1}) and cultured at 37 °C (5% CO_2). When the cells had reached about 80% confluence, they were seeded (100 μL) in 96-well plates at 4×10^4 cells/mL and treated with RPMI medium containing Gu, βcd, or Gu/βcd at 0.125, 0.25, 0.5, and 1 mM for 48 h. The RPMI medium was then discarded and replaced with DMEM without phenol red.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to assess cell viability. MTT (5 mg mL^{-1}) was deposited in each well and cultured for 3 h. Subsequently, formazan crystals were dissolved with SDS (10%) in HCl (0.01 M) for 4–18 h. Absorbance was measured with a spectrophotometer (Multiskan Spectrum; Thermo Scientific, Vantaa, Finland) at 570 nm. Cytotoxicity was estimated based on cell viability in comparison with the control group (defined as 100% cell viability), as described by Kong et al. [20]: non-cytotoxic, cell viability > 90%; slightly cytotoxic, cell viability 60–90%; moderately cytotoxic, cell viability 30–59%; and severely cytotoxic, cell viability < 30%. The data was obtained from two independent experiments carried out in sextuplicates and expressed as mean ± standard deviation. Statistical significance was determined by two-way analysis of variance and Bonferroni's post-test (α = 5%). Normal data distribution and equality of variance were assumed after the performance of Kolmogorov–Smirnov and Bartlett's tests (GraphPad Prism 5.0; GraphPad Software Inc., La Jolla, CA, USA).

Antimicrobial activity

The microdilution method was used to assess bacterial susceptibility and was performed in accordance with the Clinical Laboratory Standard Institute protocol [21]. *E. coli* (ATCC 25922), *S. aureus* (ATCC 29213), *S. mitis* (ATCC 49456), *S. mutans* (ATCC 25175), *S. sanguis* (ATCC 10557), and *A.a* (ATCC 29522) were inoculated into BHI broth (supplemented with 1% menadione and 1% hemin for *A.a*) and incubated at 37 °C for 24 h. *S. aureus* and *E. coli* were cultured aerobically; *S. mitis*, *S. mutans*, and *S. sanguis* were cultured microaerobically; and *A.a* was cultured anaerobically.

A serial dilution of Gu, Gu/βcd, or βcd (80.6–0.04 mM) was prepared in 96-well plates with Mueller Hinton broth. Bacterial inocula, previously adjusted to 0.5 on the McFarland scale (1×10^8 CFU/mL), were added to the plates and incubated at 37 °C for 24 h. Blank wells (culture medium only) and growth control wells (bacteria plus drug-free culture medium) were also included in the plates ($n = 3$). The turbidity of each well was determined using a spectrophotometer (TP Reader; Thermoplate, Palm City, FL, USA) at 492 nm. This parameter was used to obtain minimum inhibitory concentration (MIC) values, defined as the lowest drug concentration that inhibited 90% (MIC_{90}) or 50% (MIC_{50}) bacterial growth compared with the growth control. Minimum bactericidal concentrations (MBCs) were determined 24 h after plating 2 μL content from each well on a Mueller Hinton agar Petri dish, by assessing the lowest concentration that prevented visible growth on the agar surface.

Histological observation

Forty-five male rats (*Rattus norvegicus*, albinus, Wistar) weighing about 300 g each were used for this study (ethics committee approval no. 013/2014). The animals were kept in a quiet room at 22 °C, on a 12-h light/dark cycle, at the Biomedical Sciences Institute, Universidade de São Paulo, São Paulo, Brazil. Food and water were provided ad libitum.

The rats were divided randomly into three treatment groups: positive control (C+; Alvogyl™), βcd only, and Gu/βcd inclusion complex (10 mM). On day 0, the animals were anesthetized with ketamine chloride (90 mg/kg) and xylazine (10 mg/kg, IM). The right mandibular first molar was extracted from each rat and the socket was filled with 0.1 mL of 1 mg/mL epinephrine for 5 min to inhibit blood clot formation. All rats received acetaminophen (200 mg/kg) after surgery. On day 3, dry socket was confirmed by the absence of a blood clot inside the alveolus and the empty alveolus was irrigated with sterile saline and filled with one of the three treatment materials. The rats were sacrificed in a CO_2 chamber on days 7, 14, and 21 after extraction ($n = 5$/group/day). A mandible fragment containing the socket was removed from each rat and fixed in 4% paraformaldehyde for 30 h, decalcified in 4.13% EDTA (pH 7.4) for 5 weeks, dehydrated with increasing concentrations of ethanol, and embedded in paraffin. The blocks were cut into 6-μm sections in the sagittal plane and stained with hematoxylin and eosin. An experienced histologist made a qualitative evaluation of the socket healing phases in the slides under a light microscope.

Results

Nuclear magnetic resonance spectroscopy

Figure 1A shows the bidimensional 2D-ROESY contour map for the Gu/βcd inclusion complex. The insertion of a guest molecule, Gu, in the cavity of βcd, showed shift changes in the proton of these molecules, which could be visualized by spatial correlations highlighted, between protons H-3 (close to the wider rim) and H-5 (close to the narrower rim) of internal βcd cavity and the aromatic protons (H-a and H-b) of Gu, which were coupled at short distances, smaller than 5 Å (expanded view in Fig. 1B). It was not possible to observe spatial correlation between Gu's methoxy group hydrogen and βcd hydrogens. Figure 1C shows the disposition of Gu within the βcd cavity.

Cell viability

Osteoblast viability after treatment with Gu and Gu/βcd is presented in Fig. 2. At 1 mM concentrations, Gu and Gu/βcd were moderately cytotoxic. At 0.125–0.5 mM concentrations, Gu was slightly cytotoxic. Gu/βcd was slightly cytotoxic at 0.25 and 0.5 mM concentrations, and non-cytotoxic at 0.125 mM. Gu/βcd was significantly less cytotoxic than Gu at 0.125–0.5 mM ($p < 0.001$). βcd showed no cytotoxic effect (93–104% cell viability).

Antimicrobial activity

Plain guaiacol and Gu/βcd presented antimicrobial effects against all tested bacteria, whereas βcd was not able to inhibit the growth of any of these microorganisms. Table 2 shows MIC_{50}, MIC_{90}, and MBC values for each group, suggesting that smaller concentrations of guaiacol were required to inhibit bacterial growth when the phenolic was inside the Gu/βcd complex, in comparison with plain guaiacol.

Histological observation

Figure 3 illustrates the histological aspects of sockets after treatment. By day 7, Alvogyl™-treated alveoli (C+) were

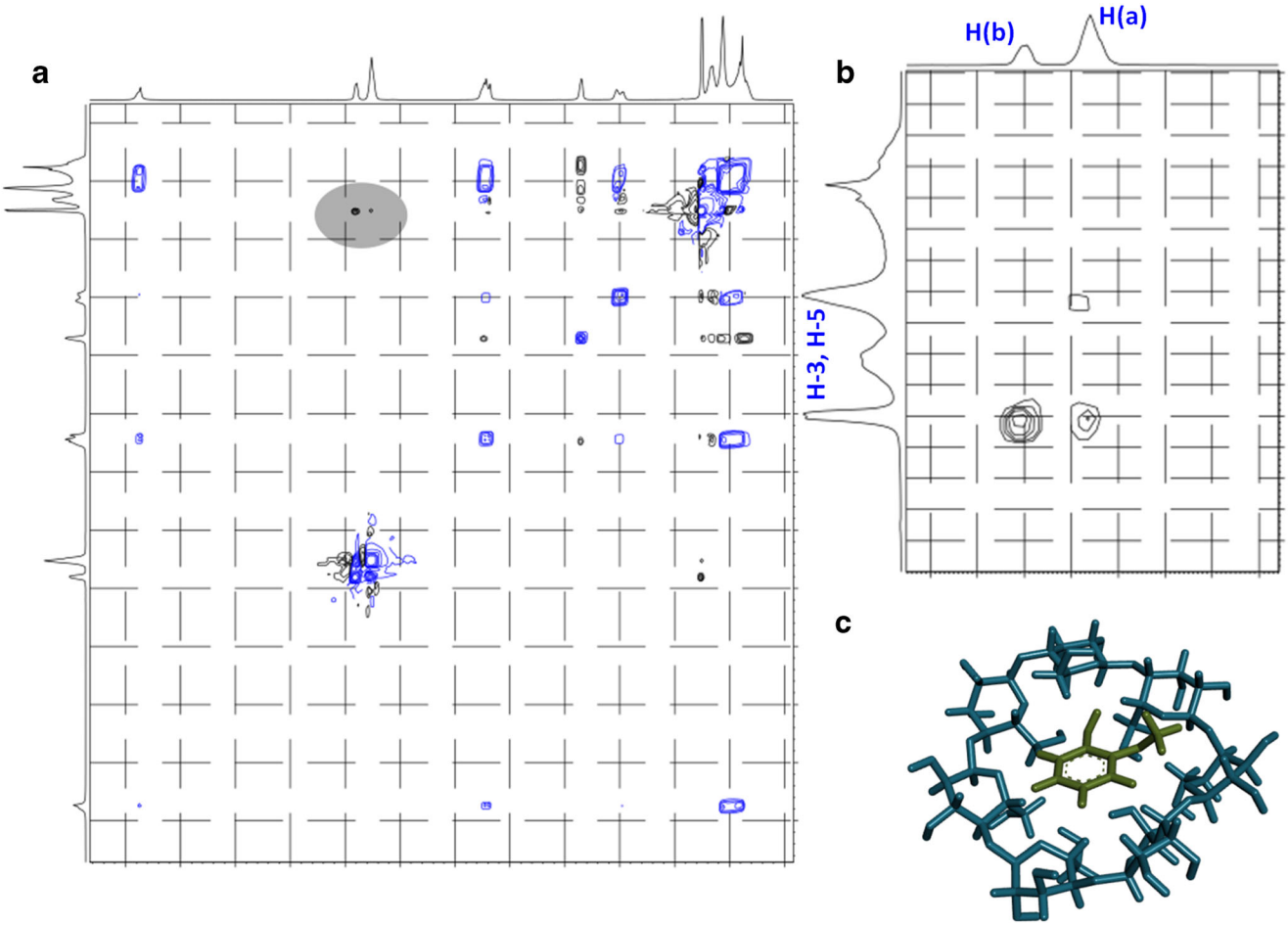

Fig. 1 (A) NMR 2D-ROESY contour map (400 MHz) obtained for the Gu/βcd complex. (B) Expanded view of region with correlations between Gu and βcd. (C) Disposition of Gu within the βcd cavity

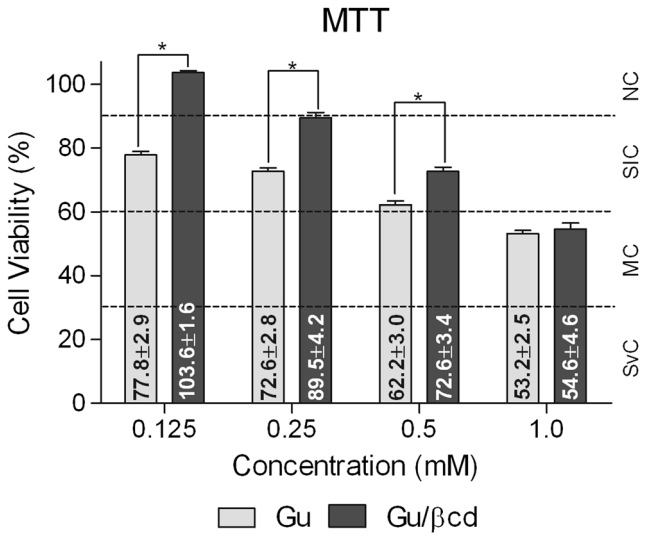

Fig. 2 Osteoblast viability (mean ± SD) obtained with the MTT assay after 48-h treatment with pure guaiacol (Gu) or the inclusion complex Gu/βcd. *$p < 0.001$ (two-way ANOVA and Bonferroni post-test). NC, non-cytotoxic; SlC, slightly cytotoxic; MC, moderately cytotoxic; SvC, severely cytotoxic

filled with loose connective tissue containing principally newly formed blood vessels, fibroblasts, and collagen fibers. Very intense inflammatory infiltrate was concentrated near remaining Alvogyl™, especially in the cervical thirds of alveoli, but also in the middle and apical thirds. Abundant osteoclasts along the alveolar bone and no bone formation were observed. On day 14, material debris remained visible. Connective tissue was predominant and little bone formation was seen in the apical region. On day 21, bone formation expanding from the socket walls to the central region was observed in the middle and apical thirds. The alveolar entrances still contained material debris, and macrophages were detected nearby. One rat developed an abscess.

Sockets treated with βcd alone were filled completely with highly vascularized connective tissue on day 7. The cervical thirds of alveoli showed severe inflammation and abundant osteoclasts along the alveolar bone. In the apical region, thin bone trabeculae and numerous osteoblasts were observed. On day 14, trabecular bone expanded to the apical and middle thirds. On day 21, areas of new bone formation were seen in the entire socket, but bone trabeculae were immature and surrounded by connective tissue. On the alveolar surface, material debris involved by inflammatory tissue remained. Two animals developed abscesses.

On day 7, the Gu/βcd group presented material remains only in the socket entrance and a region of inflammatory cells, mainly neutrophils, beneath these remains. The sockets were filled with loose connective tissue with numerous blood vessels, the proportion of which was larger at the socket apex. In the apical region, osteoblasts and newly formed bone trabeculae were observed. Osteoclasts appeared along the alveolar bone. On day 14, connective tissue was present mainly in the cervical third, and about two-thirds of the socket had been filled by bone trabeculae, which were thicker and more closely spaced at the apex. On day 21, the entire alveoli were filled with woven bone containing numerous osteocytes and blood vessels. As the newly formed bone joined the surrounding alveolar bone, the original limits of the dental socket were difficult to identify. In comparison with the other groups, the Gu/βcd group showed more bone formation and maturation (Figs. 3 and 4).

Table 2 Minimum inhibitory and bactericidal concentrations of guaiacol and Gu/βcd

Bacteria	Guaiacol (mM)			Gu/βcd (mM)		
	MIC_{90}	MIC_{50}	MBC	MIC_{90}	MIC_{50}	MBC
E. coli	5.04	2.51	10.07	5.04	1.26	10.07
S. aureus	5.04	1.26	10.07	0.63	0.31	1.26
S. mitis	10.07	5.04	20.14	5.04	2.51	10.07
S. sanguis	10.07	2.51	20.14	2.51	0.63	5.04
S. mutans	20.14	5.04	20.14	5.04	1.26	10.07
A.a	20.14	5.04	40.29	5.04	2.51	10.07

MIC_{90}, 90% minimum inhibitory concentration; *MIC_{50}*, 50% minimum inhibitory concentration; *MBC*, minimum bactericidal concentration

Discussion

In this work, Gu was complexed with βcd to improve its physicochemical stability and biological properties, prolonging analgesia, reducing alveolar contamination, and accelerating bone repair in dry socket.

NMR showed short distances between hydrogens indicating that βcd and Gu interacted in solution to form a complex that differed from the raw materials [22]. The aromatic ring of Gu may be located inside and its methoxy group projected outside the βcd cavity, as seen in previous studies [16, 17].

Microbiological tests showed that Gu/βcd had antimicrobial effects against all tested bacteria, with aerobic bacteria showing the most and anaerobic bacteria the least susceptibility. Higher Gu concentrations were required to inhibit growth of Gram-negative *E. coli* in comparison with the other aerobic bacteria, in agreement with previous observations that Gram-negative microorganisms are less sensitive than Gram-positive microorganisms due to the presence of an outer membrane that limits substance diffusion through the lipopolysaccharide layer to the cytoplasmic membrane [8, 23].

No previous study has evaluated the use of Gu/βcd as an antimicrobial agent. Several bacteria, including *E. coli*, have been shown to be sensitive to pure Gu, but MIC values were not reported [8]. Gu was also found to be active against several

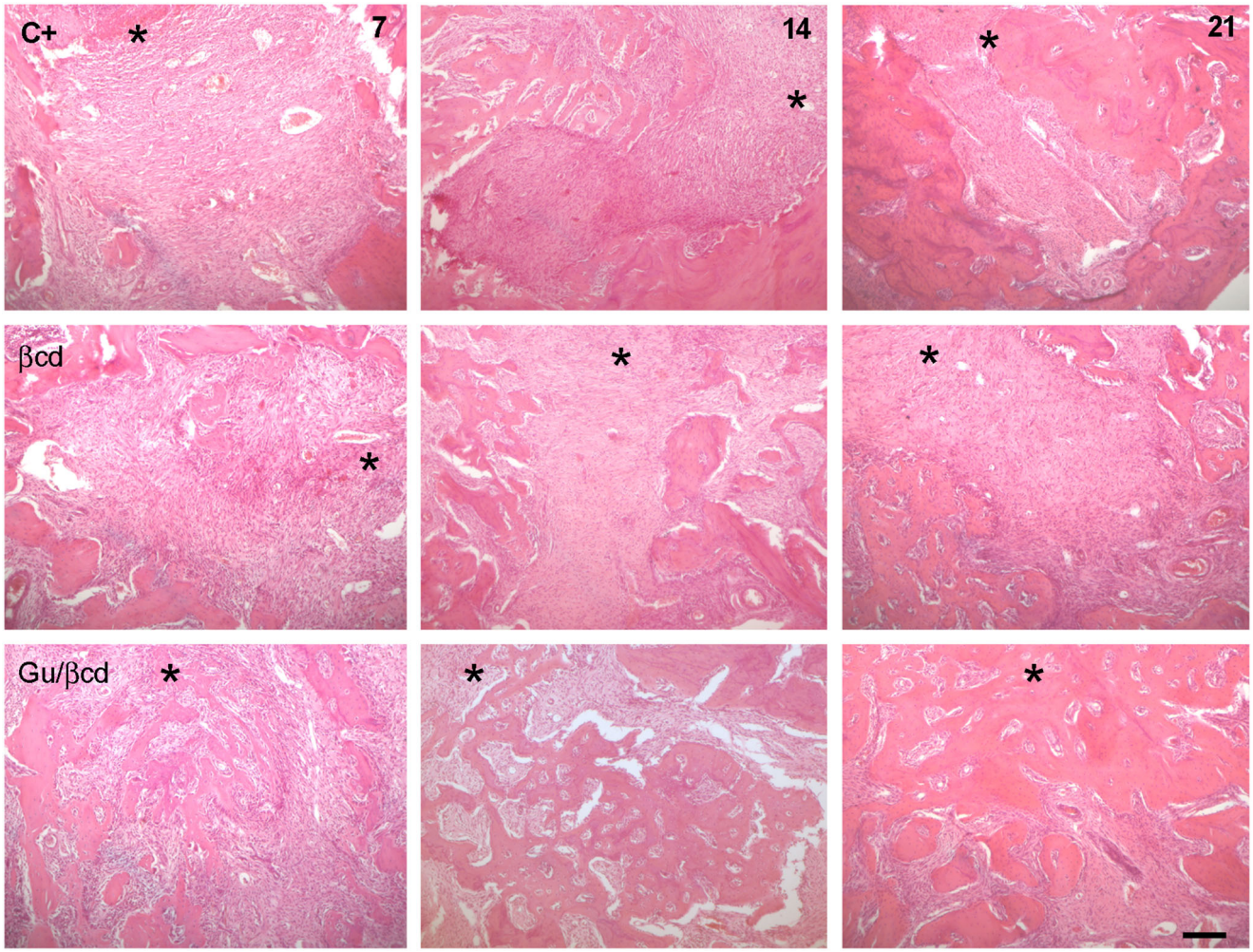

Fig. 3 Histological aspects of the apical thirds of alveoli treated with Alvogyl™ (C+), β-cyclodextrin (βcd), and Gu/βcd. Representative sections of the alveolar bone healing process at 7, 14, and 21 days post-extraction of the mandibular right molar. H&E staining, original magnification × 10. *Marks the most cervical portion of the socket on the image. Bar = 100 μm

molds and yeasts [9], suggesting that it has antifungal properties. Moreover, the complexation of Gu in βcd reduces MIC and MBC values. As βcd alone did not inhibit bacterial growth in this study, we believe it has a potentiating effect on Gu's antibacterial action, but no antibacterial effect per se.

Other studies have also demonstrated that the effects of cyclodextrin-complexed antimicrobial agents are superior to those of non-complexed agents [14, 15, 18, 24–26], perhaps due to the increased drug solubility in water, which increases contact with the microorganism [26]. Cyclodextrin should also increase drug adhesion to the bacterial cell wall because of the high affinity of OH groups localized outside the cavity that bind to bacteria via hydrogen bonds [18, 27]. This effect could also be a result of the formation of an aggregating matrix that entraps the drug, bacteria, and cellular debris and increases the degree of compound inhibition [25]. At the same time, cyclodextrin may delay drug release [24]. The mechanism by which Gu produces cytotoxic effects in animal cells is not clearly defined, but is believed to be similar to the antiseptic mechanism of action of phenolic compounds on cell membranes [11]. In our study, the highest tested concentration (1 mM) resulted in > 50% viability; cell sensitivity, however, may differ in the clinical setting, in the presence of species-related and phenotypic differences, such as adhesion, membrane permeability, and adaptive and recovery mechanisms [28]. These differences may explain the absence of local adverse effects in Haghighat et al.'s [29] clinical study, despite the use of 3% Gu (241.74 mM), a concentration more than 200 times greater than that tested in our study.

In a previous study [11], Gu had a 50% inhibition concentration (IC_{50}) of 9.8 mM in human dental pulp, with no genotoxic effect and less cytotoxicity than eugenol (IC_{50} = 0.9 mM). An assessment of colony-forming ability [10] in dental pulp cells showed that the IC_{50} of Gu was 0.78 mM and that growth inhibition levels were not related to cell death levels, as this concentration caused apoptosis in < 2% of cells. These findings suggest that Gu reduces the cell division rate, but causes little cell death. Gu was also found to be

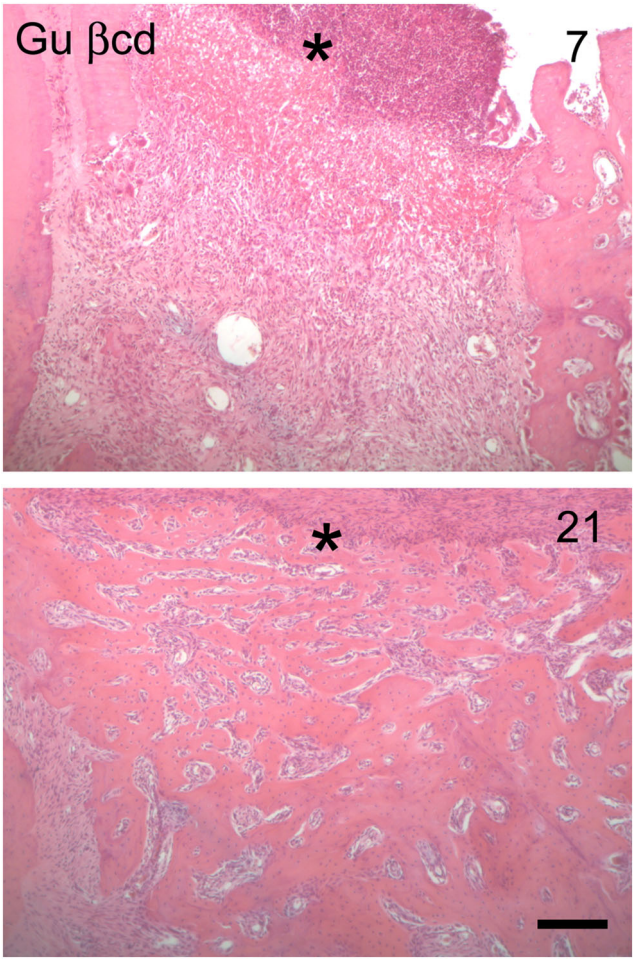

Fig. 4 Histological aspects of the cervical thirds of alveoli treated with Gu/βcd representative sections of the alveolar bone healing process at 7 and 21 days post-extraction of the mandibular right molar. H&E staining, original magnification × 10. *Marks the most cervical portion of the socket on the image. Bar = 100 μm

significantly less cytotoxic than eugenol (IC_{50} = 0.5 mM) and zinc oxide (IC_{50} = 0.2 mM) [10].

Complexating Gu in βcd significantly decreased its cytotoxicity at 0.125–0.5 mM. The decreased cytotoxicity of complexed drugs is due to direct reduction of contact with biological membranes and improved chemical stability and solubility, which increase drug effectiveness in smaller doses [30, 31]. Cyclodextrins may also reduce local tissue irritation due to decreased percentages of free drug [32].

The concomitant presence of cyclodextrin's protective effect on eukaryotic cells and damaging effect on prokaryotic cells may be explained by structural differences in the biological membranes that interact with cyclodextrin's external surface [14]. The eukaryotic cell membrane has highly organized regions called lipid rafts, where hydrogen bonds are numerous [33]. The prokaryotic cell membrane is composed mainly of phospholipids held together by van der Waals interactions, which are considerably weaker than hydrogen bonds in lipid rafts; this structure is responsible for the packaging loss of prokaryotic membranes and regions outside of lipid rafts in eukaryotes [14]. Our histological evaluation of the effects of the Gu/βcd complex on socket healing in rats differs from other approaches [34–36] because we used molar, rather than central incisor, alveoli. Although the anterior region is easier to manipulate, the rat incisor grows and erupts continuously, and the arrangement of its periodontium does not resemble that of human teeth [37]. Notwithstanding the chronological differences, our model could better represent the human healing process. Alveolar healing begins with an inflammatory phase in response to tissue damage caused by extraction, regardless of the presence of dry socket [36, 38]. All groups in our study showed inflammatory infiltrate on day 7, which decreased over time, except in groups with more abundant material remains (especially the C+ group) and in cases of abscess formation (in the βcd and C+ groups). Alveoli were filled with connective tissue containing numerous fibroblasts, newly formed blood vessels, and inflammatory cells on day 7, consistent with previous findings [36, 38]. We observed the onset of osteogenesis in the apical region, except in the C+ group, in which bone formation was less evolved.

At all evaluated timepoints, bone formation was most intense and mature in the Gu/βcd group. The repair chronology observed with Gu/βcd treatment was very similar to that described in normal alveoli [38]. Given the lack of a quantitative parameter in histological evaluation, a study limitation, and differences in experimental models (mainly related to tooth selection and evaluation times), comparison of the wound healing chronology in this study with those described in other reports [34, 35] was difficult.

Alvogyl™, a well-known material containing eugenol, iodoform, and butamben, was used as a positive control in this study. The C+ group showed increased inflammatory infiltrate and delayed bone formation at all evaluation timepoints, likely due to the persistence of material debris inside the alveoli, which can accumulate abundant microorganisms, generate foreign body reactions, and promote marked inflammation. Several clinical studies, reviews, and case reports have documented similar delayed healing and increased infection risk [1, 3, 4, 39, 40], suggesting that Alvogyl™ is not an ideal material for a control group in further studies because many other treatment modalities could be found as preferable.

In our study, remains of Gu/βcd were not found in the same quantity and depth than Alvogyl™ rests. No studies about the resorption kinetic aspects of the Gu/βcd were found to explain this phenomenon; however, we believe this may occur because the mechanical ejection and dilution of this material are easier than those of Alvogyl™ due to βcd, which is a water-soluble molecule and can also increase guaiacol's solubility [15].

The only advantage of Alvogyl™ over other treatments seems to be the great pain relief provided in the first hours of usage; after the first day, this product loses its effect and becomes inferior to other treatments [1, 4]. The delayed course

of alveolar healing and abscess formation (despite the antimicrobial components of Alvogyl™) observed in the C+ group suggests that this material loses its properties earlier than Gu/βcd, permitting microorganism accumulation.

Abscess formation and slower bone growth compared with the Gu/βcd group were observed in the βcd group. These findings confirm the antimicrobial action of Gu in vivo and its positive effect on wound healing. In addition, complexation with βcd may prolong the release of Gu. The osteogenic effect of Gu, observed by Kato et al. [6] in vitro, may also have promoted bone formation in the Gu/βcd group. However, further studies are needed to verify whether the osteogenic rate promoted by Gu treatment is clinically relevant, and whether this effect persists after complexation with βcd. The confirmation of osteogenic activity provided by Gu/βcd would have implications for its application not only in the treatment of dry socket but also in tissue engineering and socket healing before placement of dental implants. One of the study limitations is the absence of a test to prove the permanence, increase, or decrease of the analgesic effect of guaiacol after its complexation with βcd. This property is an important quality of a socket dressing material and may be assessed in future studies.

The advantages of Gu/βcd over other currently available treatments for dry socket include a good antimicrobial activity at low doses, reduced cytotoxicity compared with other well-known active molecules (e.g., eugenol and zinc oxide), promotion of appropriate alveolar healing, and the slow release of the active principle, which permits less-frequent changing of the intra-alveolar dressing and thereby reduces the number of dental visits.

Within the limitations of this animal study, the benefits gained from the inclusion of Gu in βcd have been demonstrated by the improvement in Gu's antibacterial activity and reduced cytoxicity in vitro, in addition to the achievement of an appropriate alveolar repair in vivo. Thus, Gu/βcd can be considered as an encouraging future alternative for the treatment of dry socket.

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Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted.

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