Effects of Antiseptic Solutions Commonly Used in Dentistry on Bone Viability, Bone Morphology, and Release of Growth Factors

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Purpose: Antiseptic solutions are commonly used in dentistry for a number of sterilization procedures, including harvesting of bone chips, irrigation of extraction sockets, and sterilization of osteonecrotic bone. Despite its widespread use, little information is available regarding the effects of various antiseptic solutions on bone cell viability, morphology, and the release of growth factors.

Materials and Methods: The antiseptic solutions included *1)* 0.5% povidone iodine (PI), *2)* 0.2% chlorhexidine diguluconate (CHX), *3)* 1% hydrogen peroxide (H_2O_2), and *4)* 0.25% sodium hypochlorite (HYP). Bone samples collected from porcine mandibular cortical bone were rinsed in the antiseptic solutions for 10 minutes and assessed for cell viability using an MTS assay and protein release of transforming growth factor (TGF-β1), bone morphogenetic protein 2 (BMP2), vascular endothelial growth factor (VEGF), interleukin (IL)-1β, and receptor activator of nuclear factor κB ligand (RANKL) using an enzyme-linked immunosorbent assay at 15 minutes and 4 hours after rinsing.

Results: After antiseptic rinsing, changes to the surface protein content showed marked alterations, with an abundant protein layer remaining on CHX-rinsed bone samples. The amount of surface protein content gradually decreased in the following order: CHX, H_2O_2, PI, and HYP. A similar trend was also observed for the relative cell viability from within bone samples after rinsing, with up to 6 times more viable cells found in the CHX-rinsed bone samples than in the HYP- and PI-rinsed samples. An analysis of the growth factors found that both HYP and PI had significantly lower VEGF and TGF-β1 protein release from bone samples at 15 minutes and 4 hours after rinsing compared with CHX and H_2O_2. A similar trend was observed for RANKL and IL-1β protein release, although no change was observed for BMP2.

Conclusions: The results from the present study have demonstrated that antiseptic solutions present with very different effects on bone samples after 10 minutes of rinsing. Rinsing with CHX maintained significantly higher cell viability and protein release of growth factors potent to the bone remodeling cycle.

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Antiseptic solutions in everyday dental practice are used for a variety of sterilization procedures. With respect to bone, antiseptic solutions have been used in the harvesting of bone chips[1] and irrigation of extraction sockets[2,3] and because of bone exposure in osteonecrotic bone.[4-6] They have also been used for various other decontamination protocols, such as antibacterial mouth rinses,[7] irrigation around implants,[8,9] and disinfection in advanced periodontal disease.[10-12] Furthermore, antiseptic sterilization of bone allografts is commonly used during sterilization procedures before bone bank storing.[13,14] Taken together, numerous situations exist in which bone is exposed to antiseptic solutions.

It has previously been demonstrated that these antiseptic solutions not have only cytotoxic effects on bacterial, but also on many eukaryotic, cells. Toxicity studies have demonstrated that povidone iodine (PI) has also a negative effect on the cell viability of epithelial cells.[15] Furthermore, it has been shown that chlorhexidine diguluconate (CHX) and sodium hypochlorite (HYP) solutions are toxic to fibroblasts[16] and that a high concentration of hydrogen peroxide (H_2O_2) is damaging to human gingival cells.[17] It must also be noted that apart from changes in viability, antiseptic solutions can also affect the regulation of gene expression of various cytokines and growth factors responsible for cell growth, osteogenesis, and inflammation.

Therefore, the aim of the present study was to assess the possible changes specifically to alveolar bone samples prepared and rinsed with 4 different concentrations of antiseptic solutions commonly used in dentistry and oral-maxillofacial surgery. All bone chips were exposed to the following 4 antiseptic solutions: *1)* 0.5% PI, *2)* 0.2% CHX, *3)* 1% H_2O_2, and *4)* 0.25% HYP. First, the control bone samples were visualized using scanning electron microscopy (SEM) for the presence of viable cells and protein content on the surface of the grafting materials. Next, the samples rinsed with antiseptic solutions were also qualitatively visualized for changes in bone surface morphology and protein content. Therefore, bone viability was quantified, and the release of growth factors, including transforming growth factor (TGF)-β1, bone morphogenetic protein 2 (BMP2), vascular endothelial growth factor (VEGF), interleukin (IL)-1β, and receptor activator of nuclear factor κB ligand (RANKL) was quantified using an enzyme-linked immunosorbent assay (ELISA) at 15 minutes and 4 hours.

Materials and Methods

ANTISEPTIC SOLUTIONS

Four antiseptic solutions were selected for the present study: *1)* 11% PI (Mundipharma Medical Company, Basel, Switzerland); *2)* 0.2% CHX (GlaxoSmithKline Consumer Healthcare, Bern, Switzerland); *3)* 2% H_2O_2 (Inselspital, Bern, Switzerland); and *4)* 5.25% HYP (Dr Speier, Münster, Germany). The antiseptic solutions were diluted in sterilized water to reach a final concentration of 0.5% PI, 1% H_2O_2, and 0.25% HYP to match previous concentrations used in clinical situations.[18-21] CHX was not diluted further. All antiseptic solutions were filtered and sterile (0.22-μm pore diameter; Merck Millipore, Billerica, MA).

BONE COLLECTION

Bone was obtained from adult pigs (Metzgerei Balsiger, Wattenwil, Switzerland), harvested from the buccal-side mandibular cortical bone using a "bone scraper" (Hu-Friedy, Rotterdam, The Netherlands), and placed into sterile plastic dishes, as previously described.[22] Ethical approval for in vivo experiments was not necessary because the pigs were killed at the local butcher for nonscientific reasons. The bone samples were then immediately collected and transported for use in the present study. In brief, bone samples harvested with a bone scraper from 1 pig were divided into 5 groups of equal weight (1 control and 4 antiseptic solution groups). Within 10 minutes, the bone was rinsed with *1)* 0.5% PI, *2)* 0.2% CHX, *3)* 1% H_2O_2, and *4)* 0.25% HYP. For each experiment, 4 independent preparations of the bone samples were prepared, and all experiments were performed in triplicate. The harvested bone samples were then exposed to the aseptic solutions for 10 minutes. Next, the bone samples were either fixed and assigned to SEM, assigned to MTS analysis for cell viability, or left in phosphate-buffered saline (PBS) solution, and the samples were collected after 15 minutes and 4 hours for protein quantification using ELISA.

SCANNING ELECTRON MICROSCOPY

The bone samples were fixed in 1% glutaraldehyde and 1% formaldehyde for 2 days for SEM. After serial dehydration with ethanol, the samples were critical point dried (Type M.9202 Critical Point Dryer; Roth & Co, Hatfield, PA) and allowed to dry overnight, as previously described.[23,24] The next day, the samples were sputter coated using a Balzers Union Sputtering Device (DCM-010; Balzers, Liechtenstein) with 10 nm of gold and analyzed microscopically using a Philips XL30 FEG scanning electron microscope to determine the surface variations among the samples.

QUANTIFICATION OF VIABLE CELLS IN BONE SAMPLES

The cell viability in each of the bone samples was determined using the CellTiter 96 One Solution Cell