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Influence of different biomaterials on the viability of *Aggregatibacter actinomycetemcomitans*

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ABSTRACT

Objectives: The aim of the present *in vitro* study was to evaluate the effects of different biomaterials used for regenerative periodontal surgery on the growth of the periodontopathogen *Aggregatibacter actinomycetemcomitans*.

Methods: Three commercially available biomaterials of synthetic origin (hydroxyapatite/beta-tricalcium phosphate, nanostructured hydroxyapatite paste, oily calcium hydroxide suspension), a bovine-derived xenograft as well as an enamel matrix derivative (EMD) were added in different concentrations to calibrated suspensions of *A. actinomycetemcomitans* ATCC 43718/33384 (serotype b/c). Equal aliquots (0.1 ml) for the viability assay were taken after 5 min, 1 h, 3 h, 8 h and 24 h, plated on blood agar and incubated in an anaerobic environment for 48 h at 37 °C. Viable cell counts were expressed as colony forming units (cfu)/0.1 ml.

Results: The results demonstrated that none of the investigated biomaterials could inhibit the growth of *A. actinomycetemcomitans* serotype b. A marked growth reduction of *A. actinomycetemcomitans* serotype c was observed in the presence of oily calcium hydroxide suspension and nanostructured hydroxyapatite. In contrast, no significant growth inhibition could be observed in the presence of hydroxyapatite/beta-tricalcium phosphate, enamel matrix derivative and bovine-derived xenograft.

Conclusions: The results of the present study suggest that none of the investigated biomaterials possesses antimicrobial properties against *A. actinomycetemcomitans* serotype b. Therefore, the use of these biomaterials for regenerative procedures should be weighted critically in the presence of *A. actinomycetemcomitans* serotype b.

1. Introduction

The major goal of periodontal therapy is to control periodontal infection and to eliminate remaining anatomic deformities by regenerating the lost periodontal supporting tissues. This process should include the formation of new periodontal ligament, new cementum with inserting periodontal ligament fibres and new alveolar bone. In order to achieve this goal, several treatment procedures have been described in the literature, including the use of bone grafts and bone substitutes, guided tissue regeneration (GTR), enamel matrix

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derivative (EMD), growth factors or combined approaches.[1–5] All of these techniques provide additional clinical improvements and radiographic bone fill in intra-osseous defects when compared with open flap debridement alone.[6] Human histological studies have demonstrated that true periodontal regeneration can be achieved with GTR as well as with enamel matrix derivative.[7,8] However, significant heterogeneity in outcome variables was observed across studies demonstrating that a complete and predictable regeneration of periodontal tissues is still difficult to obtain.[9,10] Thus, the predictability of regenerative periodontal procedures seems to be influenced by multiple factors related to the patient, defect morphology, and surgical procedures.[11] In recent years, several studies have attempted to improve the predictability of periodontal regeneration by employing minimally invasive surgical techniques,[12] combining different materials,[5,13] or evaluating the adjunctive use of local antimicrobial therapy.[14] However, one of the most important factors associated with clinical outcomes of periodontal regeneration still remains the bacterial load. Clinical evidence indicates that the presence of periodontal pathogens interferes with regeneration of periodontal tissues.[15,16] Thus, Machtei et al.[17] demonstrated a correlation between the presence of *Aggregatibacter actinomycetemcomitans* and the outcome of regenerative periodontal therapy. Colonizing periodontal pathogens can influence and disrupt the blood clot during the early healing stage and affect the process of new tissue formation. Several studies demonstrated a negative correlation between bacterial colonization of GTR membranes and the clinical attachment gain.[18,19] Furthermore, it has been shown that bacterial colonization of GTR membranes depends on the material properties, e.g. surface characteristics.[20] Thus, putative periodontal pathogens may not only damage newly formed periodontal tissues but may also interact directly with the biomaterial. Periodontal pathogens associated with severe cases of periodontitis include *A. actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*.[21]

A. actinomycetemcomitans is a small, non-motile, gram-negative coccobacillus, highly associated with aggressive periodontal disease (Fig. 1).[22] This species is characterized by leukotoxin production, the ability to invade oral epithelial cells and cause destruction to underlying tissues.[23,24] Amongst the seven currently recognized serotypes (a–g) of *A. actinomycetemcomitans*, serotypes a, b and c are most prevalent in the oral cavity.[25] The serotype b strains most properly predominate lesions of patients with localized aggressive (formerly localized juvenile) periodontitis,[26] whereas serotype a is more commonly detected in samples from chronic (formerly adult) periodontitis subjects.[26] In healthy subjects, serotype c strains are frequently observed.[27]

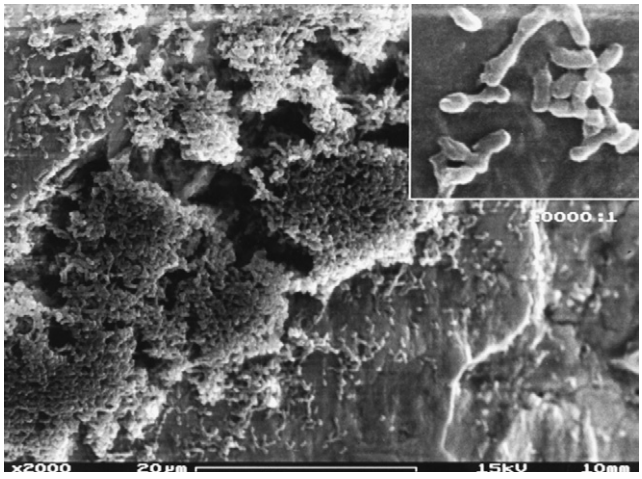

Fig. 1 – Scanning electron microscopy image of *A. actinomycetemcomitans* serotype b.

The hypothesis of this study was that biomaterials used for periodontal regeneration could modulate the growth of *A. actinomycetemcomitans*. Therefore, this study evaluates the influence of different biomaterials, routinely used for periodontal regenerative surgery, on the survival of *A. actinomycetemcomitans*.

2. Materials and methods

2.1. Biomaterials examined

Five commercially available biomaterials that are used routinely to promote periodontal regeneration were used in this study: (1) nanostructured hydroxyapatite paste (NHA) (Ostim®, Heraeus Kulzer, Germany), (2) bovine-derived xenograft (BDX) (Cerabone®, Botiss dental GmbH, Germany), (3) oily calcium hydroxide suspension (OCHS) (Osteora®, DFS-Diamon Riedenburg, Germany), (4) hydroxyapatite/beta-tricalcium phosphate (HA/β-TCP) (Bone Ceramic®, Straumann, Switzerland), and (5) enamel matrix derivative (EMD) (Emdogain®, Straumann, Switzerland).

2.2. Bacterial strains and culture conditions

The following strains of bacteria were used for the experiments: *A. actinomycetemcomitans* serotype b (ATCC 43718) and *A. actinomycetemcomitans* serotype c (ATCC 33384). The bacterial strains were grown in CASO bouillon (peptone from casein and from soy, glucose, sodium chloride, dipotassium hydrogen phosphate; Merck, Darmstadt, Germany) for 48 h at 37 °C before any assays were performed. In order to determine the total viable counts for each of the bacterial strains, serial dilutions (1:100) of each sample were prepared using sterile 0.9% sodium chloride solutions (NaCl). One hundred µl from each dilution for *A. actinomycetemcomitans* serotypes b and c were plated on Columbia agar with 5% sheep blood (Heipha, Heidelberg, Germany) and incubated in an anaerobic atmosphere enriched in 10% CO_2 (Anaeorocult®, Merck, Darmstadt, Germany) at 37 °C for up to 48 h. The bacterial concentrations were adjusted to the same densities of colony forming units per millilitre (cfu/ml) for all experiments.

2.3. Bacterial viability assay

For the experiments the different biomaterials were dissolved in sterile 0.9% sodium chloride solutions (NaCl) at different concentrations. The oily calcium hydroxide suspension was dissolved in sodium dodecylbenzene sulphonate (DBS) obtained from Sigma Aldrich (Crailsheim, Germany). Calibrated suspensions of *A. actinomycetemcomitans* serotypes b and c were incubated with different aliquots of test and control solutions