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Cytocompatibility of polymer-based periodontal bone substitutes in gingival fibroblast and MC3T3 osteoblast cell cultures

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ABSTRACT

Objectives. Inflammatory periodontal diseases are accompanied by destruction of periodontal tissue and alveolar bone. Infrabony lesions can be regenerated with adequate bone substitutes, which require high biocompatibility of the material.

Methods. To rate the biocompatibility of nine polymeric periodontal bone substitutes (Bio 1–Bio 9), cell viability and cytotoxicity assays were performed. For viability, human gingival fibroblasts (HGFs) and MC3T3 osteoblasts were cultured on the bone substitutes. For cytotoxicity, biomaterial extracts were prepared by incubation with culture medium for maximally 28 days, and cells were exposed to the extracts for 1 day. Polymers Bio 1 to Bio 5 were prepared by solvent casting, Bio 6 to Bio 9 by photopolymerization of the monomers at wavelengths of 400–500 nm in the presence of a suitable photoinitiation system.

Results. Bio 1, Bio 3, Bio 4, Bio 5, and Bio 7 showed moderate to excellent cytocompatibility for both HGFs and osteoblasts in viability tests. Together with the results of the cytotoxicity assays, four of the nine tested polymers were considered cytocompatible: Bio 1 (poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate; PVB)), Bio 4 and Bio 5 (functionalized oligolactones), and, to a limited degree, Bio 7 (urethane methacrylate). Except for Bio 7, the cytocompatible polymers showed intermediate water contact angles (74–85°) and therefore moderate to low hydrophilicity.

Significance. The non-cross-linked polymers Bio 1, Bio 4, or Bio 5, and the photopolymerized polymeric network Bio 7 display good/excellent cytocompatibility and are therefore potential candidates for tissue engineering in alveolar bone substitution.

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1. Introduction

Periodontitis is an inflammatory disease accompanied by destruction of periodontal tissue and alveolar bone, hence leading to the formation of periodontal pockets and, in severe cases, tooth loss [1]. Therefore, a successful therapy of periodontitis aims at regaining attachment of the loosened teeth and comprises both basic anti-infectious therapy and directed regeneration of the tooth-supporting apparatus [2,3]. For this reason, restoration and maintenance of bone by periodontal tissue engineering has become increasingly important [4–7]. The most widely used treatment modalities for regeneration of periodontal osseous defects are bone replacement grafts, including autologous grafts from intraoral donor sites, allografts, xenografts, and alloplastic bone substitutes [8–13]. However, donor site morbidity and constraints on obtainable quantities limit the use of autologous bone grafts. In addition, disadvantages of freeze-dried bone allo- and autografts, such as complications in harvesting, immunogenic rejection, and risk of disease transmission remain unsolved issues [14].

Topical alloplasts clinically utilized in regenerative periodontal treatment are calcium phosphate ceramics (tricalcium phosphate, hydroxyapatite), calcium sulfates, bioactive glasses, glass ionomers and polymers, sometimes also in combination with bone morphogenetic proteins (BMPs) or other bone growth promoting factors [15–17]. Different kinds of polymeric biomaterials have already been employed for bone tissue engineering [18], which can be divided into natural polymers (*e.g.* collagen and fibrin) and synthetic polymers (*e.g.* poly(lactid acid) – PLA; poly(glycolic acid) – PGA) [19–21]. In general, common disadvantages of clinically applied bone substitutes are: intricacy of application, postoperative egression of the graft from the bone defect, insufficient support of the loosened teeth, and long time periods for bone regeneration [22].

Therefore, the present *in vitro* study was designed with the aim to develop pre-formable, injectable, and *in situ*-hardening synthetic alloplastic materials for periodontal bone replacement, which are suitable for the filling of complex bone defects, form a close contact with the surrounding tissue, and thus provide immediate stabilization of loosened teeth. In this context, non-degradable polymeric biomaterials are likely to provide long-term physical stabilization, whereas biodegradable polymeric materials, in addition to initial physical support, are expected to be gradually replaced by the host's bone to provide reattachment to the mobile teeth.

Alloplastic materials differ in composition, biocompatibility and resorption characteristics, as well as in pore density, permeability, and durability. A main attribute of synthetic bone grafts should be osteoconduction, which represents the ability to support the growth of bone [23]. Accordingly, synthetic materials should mimic the natural milieu and improve the ingrowth and proliferation of osteoblasts, as well as the sprouting of blood vessels [23–28]. In the development of new synthetic bone grafts, *in vitro* assays with cell cultures are the first step *en route* to determine the biocompatibility of a material [29–31].

In the present study, *in vitro* tests were performed for nine potential polymeric alveolar bone substitutes from different groups of polymers, which differed regarding their compositions, processing, and application properties (Tables 1 and 2). None of these polymers has been previously applied as a bone substitute. To our knowledge, the synthetic PVB (Bio 1), the biopolymer Shellac (Bio 2), and the semisynthetic oligo-L-lactide-grafted dextran (Bio 3) have never been used as a biomaterial, whereas polylactides comparable to the polymers Bio 4 and Bio 5 are widely used in medicine as resorbable sutures, osteosynthesis materials (pins, screws, and plates) or drug delivery reservoirs [32]. Methacrylate polymers containing similar structures as the ones used in this study (Bio 6–Bio 9) are currently used in dentistry as dental adhesives or dental filling materials, and, in addition, may also show potential as a tissue adhesive for different types of hard and soft tissue [33].

Bio 1 to Bio 5 are non-cross-linked polymers with thermoplastic properties soluble in various organic solvents. They can be pre-formed into films, membranes or three-dimensional devices of more complex shapes by solvent- or melt-based processing techniques.

The synthetic vinyl polymer Bio 1 is widely stable against hydrolytic and enzymatic attacks under physiological conditions [34] and the natural polyester shellac (Bio 2) is only slowly degraded upon contact with water and oxidizing agents [35]. Bio 3 to Bio 5, in turn, readily undergo hydrolytic degradation [36–38].

On the other hand, Bio 6 to Bio 9 are cross-linked polymers produced by photopolymerization of the corresponding monomers. Because the monomers are liquids or viscous oils at room temperature, they can be combined with further components (initiators, fillers, *etc.*) and applied as injectable formulations to fill up bone defects. The biomaterials are prepared by radical polymerization either thermally at body temperature or photochemically by irradiation.

Bio 6 to Bio 9 vary in their degradation behavior from slow (Bio 6) over intermediate (Bio 8) to rapid degradation (Bio 7 and Bio 9) [39,40].

In the present study, degradable polymeric biomaterials (Bio 3–Bio 5 and Bio 7–Bio 9) were analyzed, which are ideal for periodontal bone substitution and subsequent gradual replacement by the host's bone if they show high cytocompatibility, proper applicability as a shapeable paste, film or gum, and a sufficient mechanical strength after hardening. Alternatively, non(slowly)-degradable biomaterials were tested (Bio 1, Bio 2 and Bio 6) which are assumed to permanently physically stabilize the teeth and thus may be advantageous in patients with a low bone regeneration capacity due to their age or general state of health.

Two cell types were selected for the *in vitro* tests, which are directly exposed to the filler materials *in vivo* and also affected by the inflammatory processes during periodontitis: human gingival fibroblasts (HGFs) and MC3T3 osteoblasts. To determine the cytocompatibility of the materials, cell viability and cell cytotoxicity assays were chosen, which are well-established methods for *in vitro* studies of bone substitutes [27,29,41]. The main goal of the study was to identify biocompatible bone substitutes for alveolar bone replacement in the periodontal lesion.