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Socket augmentation using a commercial collagen-based product — an animal study in pigs

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ABSTRACT

The aim of the present study was to identify properties of pure collagen for augmentation techniques and compare to a proved xenogenic material and natural bone regeneration. For that the osteogenesis of extraction alveoli after augmentation with a collagen cone covered with an absorbable collagen membrane in a single product (PARASORB Sombrero®, Resorba) was evaluated in a pig model. Extraction alveoli were treated with the collagen cone and the collagen membrane in a single product (test group; n = 7) or demineralized bovine bone mineral and a collagen membrane (two separate products; positive control; n = 7). Untreated alveoli were used (n = 6) as negative controls.[1] Bone specimens were extracted 1 and 3 months after teeth extraction. Serial longitudinal sections were stained with Masson Goldner trichrome. Furthermore, bone specimens were examined using X-ray analyses. Significant differences of bone atrophy were detected 12 weeks after material insertion using X-ray analyses. The bone atrophy was reduced by approximately 32% after insertion of the positive control (P = 0.046). Bone atrophy reached 37.6% of those from untreated alveoli (P = 0.002) using the test group. After 4 weeks, bone formation was noticeable in most sites, whereas after 12 weeks of healing, specimens of all groups exhibited nearly complete osseous organization of the former defected area. The mandibulary bone texture showed typical spongious bone structures. Histomorphometric analyses revealed after 4 and 12 weeks significant higher levels of bone marrow in test and negative control than in positive control. Quantification of bone tissue and osteoid does not show any significant difference. The present study confirms reduced bone resorption following socket augmentation with an absorbable collagen membrane with collagen cone while the resulting bone structure is similar to natural bone regeneration. Pure collagen can be used for bone augmentation, and shows over other xenogenic materials, a clear advantage with respect to the bone density and structure.

1. Introduction

Fresh extraction sockets of the alveolar ridge represent a special challenge due to the fact that the normal healing process of an extraction socket is one of regressive remodeling. After tooth extraction alveolar bone resorption is accelerated most significantly over the first several months [1,2]. Today, teeth replacement with implant-supported prostheses is a predictable option of therapy. The buccal wall is particularly important for implant placement and its loss can lead to un-esthetic gingival discoloration, peri-implantitis, thread exposure, and implant failure [3,4]. Thus, there is a high interest in minimizing tissue resorption after tooth extraction and maintaining the contour of the alveolar crest. Bone resorption generally and especially at the buccal wall has been reduced but not prevented when using bone augmentation materials [5]. Using post-extraction ridge preservation procedure, 85% of the initial ridge dimensions could be preserved [6].

Autogenous bone still is the gold standard for the reconstruction of bone defects and augmentations, due to its osteoconductive and osteoinductive properties. However, the amount of autogenous graft that can be harvested is limited. Therefore, various bone substitution materials have been used for studying ossification and bone formation in order to enhance alveolar ridge dimensions after tooth extraction.

Different types of biomaterials, such as minerals and non-mineral based materials as well as natural and artificial polymers have been introduced. Allogenic, xenogenic, or alloplastic bone grafts are mostly static, inert materials and often found un-resorbed in graft sites. For a long time, the goal of many studies on the bone healing was completely

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[1] Abbreviations: CC = collagen cone; DBBM = demineralized bovine bone mineral; NC = negative control; PC = positive control.

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resorbable biomaterials. Though, it has been shown that a high rate of resorption could have an effect on new bone formation as the bone substitute was degrading faster than a new bone tissue was built up [7,8]. Furthermore, residual graft particles that do not resorb can interfere with stress- and strain-induced bone remodeling. For that, a number of natural and synthetic biodegradable polymers are in use as tissue scaffolds. Natural polymers used in bone tissue engineering include collagen, fibrin, alginate, silk, hyaluronic acid, and chitosan [9].

Collagen is physiologically ubiquitous, and the most abundant extracellular matrix protein and component of connective tissue in the human body. In the form of elongated fibers, collagen is found in tendon, ligament and skin, as well as in cartilage and bone. This natural polymer is commonly used in medicine, dentistry, pharmacology, cosmetology and tissue engineering applications because of its excellent biocompatibility, low antigenicity, high biodegradability, and good hemostatic as well as cell-binding properties [10,11]. It is well known that collagen undergoes rapid degradation upon implantation within 4–5 weeks [12]. Collagen can be used in various forms, e.g. gels, sponges, membranes, scaffolds or powder [10]. The diversity of forms allows collagen to be efficient in various fields, including wound healing, and soft and bone tissue augmentation. Recently, mesenchymal stem cell osteogenic differentiation as well as alveolar ridge augmentation was demonstrated using collagen scaffolds [12,13].

Histological outcome of augmented areas has been proven to be very unsteady and has to be classified into stages of bone regeneration even in exact time controls [14–16]. The reasons for that could be age, genetics, and metabolism, and have to be identified in the future to ease the choice of augmentation procedure and time decision for the second surgery of each patient.

The aim of the present study was to identify properties of collagen for augmentation techniques and compare to a proven xenogenic material and natural bone regeneration. For that, osteogenic potential of a collagen cone covered with a collagen membrane in a single product was examined in fresh extraction sockets of a well-documented animal model. The osteogenic potential of the collagen cone was compared with those of un-augmented sockets and deproteinized bovine bone mineral (DBBM) treated extraction sockets covered with a collagen membrane. The histological analysis focused on alveolar bone resorption as well as on amount and quality of new bone formation around the different grafting materials.

2. Material and methods

2.1. Bone substitution materials

2.1.1. PARASORB Sombrero® (Resorba Wundversorgung GmbH, Nürnberg, Germany)

PARASORB Sombrero® is a combination of an absorbable collagen membrane and an absorbable collagen cone in a single product. Both components – membrane and cone – are firmly connected together for easy and reliable handling. They consist of an equine type 1 collagen (31.2 mg) without chemical additives or cross-linking agents, manufactured according to a very special procedure (complete reconstitution of collagen). The dense nature of the membrane component prevents ingrowth of connective tissue and thus guarantees a reliable barrier function, as well as closure sealed against saliva. The special surface microstructure allows growth coverage with bone-forming cells, as well as rapid epithelization above the membrane (Resorba Medical GmbH 2014).

2.1.2. Bio-Oss® (Geistlich Pharma AG, Wolhusen, Switzerland)

Geistlich Bio-Oss® is a natural bone mineral originated from cattle. The granules of spongious bone are produced in a multi-stage purification process. This material is chemically and structurally (macro- and microporous) comparable to mineralized human bone (Geistlich Pharma AG 2011).

2.1.3. Bio-Gide® (Geistlich Pharma AG, Wolhusen, Switzerland)

Geistlich Bio-Gide® is a resorbable collagen membrane with a bilayer structure. The membrane consists of natural collagen obtained from pigs without further cross-linking or chemical additives. The porous surface of the membrane allows for the ingrowth of bone-forming cells. The dense surface prevents the ingrowth of fibrous tissue into the bone defect (Geistlich Pharma AG 2011).

2.2. Animal model

The protocol of the study was approved by the Commission for Animal Studies of Western Pomerania, Germany (LALLF M-V/TSD/7221.3-1.1-012/11). The study was performed on 20 15 month-old domestic pigs (female, about 160 kg). The pigs were randomly distributed into 6 groups according to the different healing periods and inserted materials (Table 1).

2.3. Anesthesia

The extractions, implantations, and euthanasia were performed under general anesthesia under the surveillance of a veterinarian. Anesthesia was induced by intravenous injection of 2 mg/kg body weight azaperon (Stresnil®, Janssen-Cilag, Germany) and 15 mg/kg ketamine (Ursotamin, Serumwerk Bernburg, Bernburg, Germany). To reduce salivation, 0.02 mg/kg atropine (B. Braun Melsungen AG, Melsungen, Germany) was administered. For infection prophylaxis, 3 ml/kg of Veracin®-compositum (Albrecht GmbH, Aulendorf, Germany) were injected intramuscularly. The analgesia and antiinflammation were performed by administration of Flunidol RP (0.08 mg/kg i.m., CP-Pharma, Burgdorf, Germany) intramuscularly.

2.4. Surgical interventions

In a split-mouth design, both mandibles of each pig were treated exactly the same way. For that both permanental P3 premolars have been extracted in all animals. Care was taken to avoid the fracture of bone walls (Fig. 1A). After removal of the premolars extraction alveoli were treated with a collagen cone (CC; PARASORB Sombrero®, Resorba Wundversorgung GmbH, Nürnberg, Germany; Fig. 1B) or demineralized bovine bone mineral (DBBM) concomitant with the placement of a collagen membrane as positive control (PC; Bio-Oss® + Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland; Fig. 1C) as described in Table 1. Untreated alveoli served as a negative control (NC). After insertion of

Table 1
Study protocol for the insertion of bone grafting materials (split mouth).

Group number	Mandibula right side	Mandibula left side	Treatment time	Amount of animals
1	Untreated (negative control; NC)	PARASORB Sombrero® (CC)	4 weeks	3
			12 weeks	3
2	PARASORB Sombrero® (CC)	Bio-Oss® + Bio-Gide® (positive control; PC)	4 weeks	4
			12 weeks	4
3	Bio-Oss® + Bio-Gide® (positive control; PC)	Untreated (negative control; NC)	4 weeks	3
			12 weeks	3