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Biocompatibility of MgF_2-coated MgNd2 specimens in contact with mucosa of the nasal sinus – A long term study

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ABSTRACT

Up to now, different surgical techniques and stent systems have already been developed and tested for the continuous and adequate ventilation of the frontal sinuses. However, the results achieved still remain poor. Magnesium-based implants have been successfully used in numerous clinical applications. Offering excellent biocompatibility and biodegradability it may be the ideal material for the development of novel implants of the nasal sinus. Here, we present for the first time results on the behaviour of magnesium alloy in a unique environment, i.e. in contact to the nasal mucosa, air and nasal secretion. In a prospective longitudinal study, magnesium fluoride-coated MgNd2 specimens were implanted in the frontal sinuses of 12 minipigs for the investigation of biocompatibility and of the interface between the implant and the mucosa. Endoscopic examinations, histopathological evaluation and EDX measurements were performed regularly up to 180 days. Endoscopic evaluation showed focal mucosal reaction, however, without affecting the patency of the sinus. In addition, no signs of bacterial infections were observed. The EDX analyses showed a marginal but steady increase in the Mg concentration in the mucosa over 180 days. Histological analysis revealed a locally confined moderate mucosal hyperplasia and unspecific inflammatory reaction. Furthermore, we did not find any osteoinductive effects of the magnesium alloy. The results indicate the excellent biocompatibility of the MgNd2 alloy in contact with nasal mucosa and provide a novel material compound and solid proof-of-principle for the development of magnesium-based nasal stents.

1. Introduction

Chronic sinusitis is defined as inflammation of the paranasal sinus system persisting for more than 12 weeks [1]. Internationally, the prevalence of chronic rhinosinusitis is about 5% (range: 1–9%). There is a major focus on nasal polyposis [2–5]. Furthermore, multicentre studies in the United States have shown that even acute sinusitis and its medication represent a considerable socioeconomic burden, with overall costs of $2.2bn per annum [6]. The treatment of chronic sinusitis entailed global costs of $4–6bn in the 1990s, with individual costs of up to $1200 per patient, per annum [5,7–10].

Despite conservative therapy options with topical and systemic corticosteroids, antibiotics and antimycotics [11,12], most patients have to undergo surgical treatment/removal of the focus [1]. In a retrospective study by Baumann et al., treatment of the paranasal sinuses was carried out in 85% of 45,000 inpatients with chronic sinusitis [13]. In the literature, however, the recurrence rate for chronic, polypoid sinusitis following operative treatment is given as 20–60% [12,14,15]. The probability that revision surgery will be needed is stated as 20% after an observation period of 5 years [16]. A publication by Hosemann et al. indicates a 50% restenosis rate where the neo-ostial diameter is 2 mm, and a 16% restenosis rate where the diameter exceeds 5 mm [17]. Previous therapeutic approaches such as topical cortisone application [18], innovative surgical techniques [19,20] and the surgical insertion of a placeholder (e.g. a silicone, gold or nitinol stent [21,22]), augmented by drug delivery systems [23], did not yield lasting therapeutic success. Although the insertion of a silicone stent ensures ventilation, high rates of neo-ostial restenosis were observed at intervals where the stent was removed after less than 6 months [22]. In a study by Yamasoba et al., good outcomes were achieved by inserting a silicone T-tube in the frontal sinus where the T-tube

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remained in place for more than 6 months [24]. Additionally, stent displacements and bacterial colonisation of surfaces have been detected on the implanted material over time [25]. Neel et al. observed that, unlike flexible systems, rigid placeholders prevent re-epithelialisation and promote fibroplasia with scar formation [26]. Despite the development of many novel stent technologies for the paranasal sinus region, no stent has yet become established in routine clinical practice. Based on this experience, work is currently being done on biocompatible materials and stent systems to ensure long-term ventilation, and to prevent bacterial superinfections and foreign-body reactions to materials used.

The use of biocompatible, degradable materials (and combinations thereof) in the human body is a possible alternative to previously available, non-degradable implants. The envisaged benefits of biocompatible, resorbable materials are reduced local foreign-body reactions, antibacterial action caused by the material and its surface coatings, and complete material degradation. To date, resorbable materials like polymers [27,28] have been tested in animal experiments, primarily in orthopaedics (screws, intramedullary nails, osteosynthesis plates) and in cardiovascular medicine (vascular stents) [29–32].

It was in the early 19th century that magnesium was first used as a resorbable suture material in injuries to muscle tendons and to nerves [33,34]. Magnesium was not, however – owing to considerable gas production and its rapid degradation – used in clinical practice. Only in the 21st century has magnesium (and particularly its alloys) regained importance, namely in osteosynthesis and vascular medicine. Here, in addition to good compatibility, progressive degradation is evident as a function of the material's physical properties, of geometry and of the physiological milieu [29–31,35,36]. Witte et al. found that, in addition to favourable biocompatibility, magnesium has good cell adhesion [37,38]. Gas formation in the implant environment was also observed, although no further negative local effects were apparent [39]. Another study described how, during degradation, substances including pH-dependent hydroxyapatite are deposited [40]. As far as the authors are aware, the subsequent degradation and removal of these degradation products is yet to be investigated further. To further slow down the degradation of material, other techniques have been devised, such as surface coating with fluoride. Drynda and Seitz et al. observed that a magnesium fluoride alloy resulted in slower and more homogeneous material corrosion than did a pure magnesium alloy [41,42].

The aim of this *in vivo* study is – for the first time – to test, by means of animal experiments, a MgNd2 alloy at the nasal mucosa to evaluate biocompatibility and to investigate the interface between the material of the specimen and the target tissue. Further aims are to investigate the osteoinductive effect of a fluoride-coated MgNd2 implant and the gas formation caused by magnesium alloys described in the literature. The findings may serve as a basis for developing a degradable and compatible stent system for use in the paranasal sinus system to treat chronic sinusitis in humans.

2. Methods

In this study, the biocompatibility of a total of 50 fluoride-coated magnesium-neodymium (MgF_2-coated MgNd2) specimens at the nasal mucosa of the frontal sinus in minipigs ($n = 12$) was tested for up to 180 days. Endoscopic examinations of specimens and surrounding mucosa with photographic documentation were carried out at intervals of 45 days. Histological analyses of the mucosal tissue and SEM/EDX measurements were performed after euthanasia 45, 90, 135 and 180 days following implantation, respectively.

2.1. Fabrication of magnesiumfluoride-coated MgNd2 specimens

The specimens were fabricated from a magnesium alloy containing 2% neodymium. The alloy had been produced from neodymium with a purity of 99.5% (Treibacher Industrie AG, Althofen, Austria), and pure magnesium with a purity of 99.9% (Magnesium Elektron UK, Manchester, UK). Whereas magnesium melts at 650 °C, neodymium has a melting point of 371 °C higher. In order, despite this, to extract the neodymium from the molten magnesium without evaporation of the low-melting-point magnesium occurring, a master alloy was first made with 40 wt.% neodymium by means of a casting process. Subsequently, the required quantities of the master alloy and the pure magnesium were used to create the main cast. Because of magnesium's reactivity, both casting processes were carried out in an inert argon atmosphere.

The billet from the main cast was then processed further, by means of direct extrusion, to create a final diameter of 10 mm. The billet was first turned to produce a diameter of 120 mm and shortened to remove impurities, as well as any cavities and pores. The extrusion process took place at a temperature of 350 °C by means of a ram speed of 1.4 mm/s. The rods created cooled at room temperature once the extrusion process had ended.

The geometric form selected for the specimens to be implanted was a cylinder with a diameter of 5 mm and a height of 5 mm, which had previously been turned from the 10-mm-thick rods. In order to allow the specimen to be secured at the implantation site with suture material, two overlapping through-holes were drilled in the specimen, each with a diameter of 1 mm (Fig. 1). Inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of the alloy used indicated that the proportion of neodymium was 1.7 wt.%. To reduce the corrosion rate, the specimens were also magnesium fluoride coated. In order to ensure this process produced a sufficiently thick coating, the specimens were initially boiled for 120 min in NaOH (200 g l^{-1}; Carl Roth GmbH & Co. KG, Karlsruhe, Germany). The specimens were then immersed for a further 96 h in 40% hydrofluoric acid (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) to produce a MgF_2 coating, according to Seitz et al. with an assumed uniform layer thickness of approximately 1.6 μm [42].

The specimens were sterilised in a non-destructive process by means of gamma (γ) radiation (cobalt-60) with an overall dose of 29.7 kGy (BBF Sterilisations service GmbH, Kernen-Rommels hausen, Germany).

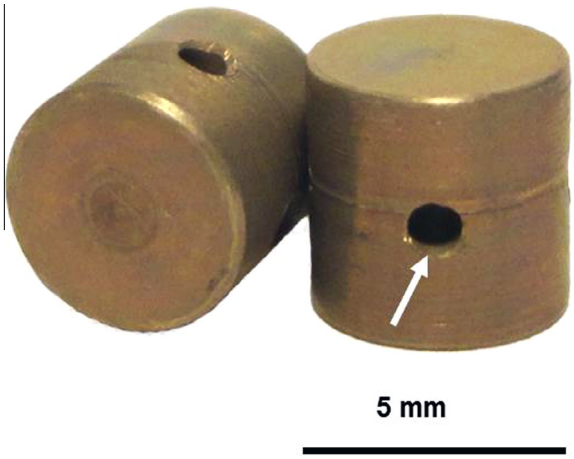

Fig. 1. MgF_2-coated $MgNd_2$ specimen; with perforation for tissue fixation (white arrow).