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Assessment of a polyelectrolyte multilayer film coating loaded with BMP-2 on titanium and PEEK implants in the rabbit femoral condyle

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ABSTRACT

The aim of this study was to evaluate the osseointegration of titanium implants (Ti-6Al-4V, noted here TA6V) and poly(etheretherketone) PEEK implants induced by a BMP-2-delivering surface coating made of polyelectrolyte multilayer films. The *in vitro* bioactivity of the polyelectrolyte film-coated implants was assessed using the alkaline phosphatase assay. BMP-2-coated TA6V and PEEK implants with a total dose of 9.3 μg of BMP-2 were inserted into the femoral condyles of New Zealand white rabbits and compared to uncoated implants. Rabbits were sacrificed 4 and 8 weeks after implantation. Histomorphometric analyses on TA6V and PEEK implants and microcomputed tomography on PEEK implants revealed that the bone-to-implant contact and bone area around the implants were significantly lower for the BMP-2-coated implants than for the bare implants. This was confirmed by scanning electron microscopy imaging. This difference was more pronounced at 4 weeks in comparison to the 8-week time point. However, bone growth inside the hexagonal upper hollow cavity of the screws was higher in the case of the BMP-2 coated implants. Overall, this study shows that a high dose of BMP-2 leads to localized and temporary bone impairment, and that the dose of BMP-2 delivered at the surface of an implant needs to be carefully optimized.

Statement of Significance

The presentation of growth factors from material surfaces currently presents significant challenges in academia, clinics and industry. Applying osteoinductive factors to different types of implants, made of metals or polymers, may improve bone repair in difficult situations. Here, we show the effects of an osteoinductive coating made of polyelectrolyte multilayer films on two widely used materials, titanium TA6V alloys and PEEK implants, which were implanted in the rabbit femoral condyle. We show that a too high dose of BMP-2 delivered from the screw surface has a negative short-term effect on bone regeneration in close vicinity of the screw surface. In contrast, bone formation was increased at early times in the empty spaces around the screw. These results highlight the need for future dose-dependence studies on bone formation in response to osteoinductive coatings.

1. Introduction

In the field of dentistry and orthopedics, the long-term success of implant-supported prostheses largely depends on rapid healing with safe integration into the bone. Additionally, achieving a solid and rapid osseointegration is necessary for early or immediate loading of the devices, which has strong implications for decreased patient morbidity, patient psychology and health care costs. The surface properties of the implant material are key factors for rapid and stable bone tissue integration.

Titanium and its alloys, such as Ti-6Al-4V (noted hereafter TA6V), are widely used materials to manufacture dental and joint

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prostheses, in view of their desirable mechanical properties, chemical stability and biocompatibility [1,2]. Several physical and chemical surface treatments have already been proposed to improve and/or speed up a reliable osseointegration of titanium implants with the aim of enhancing clinical performances [3–7]. Chemical modifications rely on acid etching [8], anodization [9] or chemical grafting [10] and physical treatments aim at changing the micrometer or nanometer scale surface roughness with a high degree of precision [11,12].

Synthetic polymers are increasingly used as alternatives to titanium in view of their lower mechanical properties and highly tunable properties in terms of molding, processing and *in vivo* imaging [13,14]. Poly(etheretherketone) (PEEK) is commonly used in orthopedics and spine surgery in the form of cages and screws [15]. In terms of biocompatibility and osseointegration, PEEK is considered as a bioinert material [15,16] with a low surface energy and limited cellular adhesion [17] in comparison to titanium [18]. Besides, *in vivo* studies performed using spine cages have shown that the direct contact between PEEK implants and bone was lower than with titanium cages [19]. Several strategies have been attempted to improve the biocompatibility and osseointegration properties of PEEK, by forming bulk composites with hydroxyapatite (HAP) [20,21], applying chemical treatments such as plasma [16,22] or coating implants with HAP [23,24], titanium [25], gold [26] or diamond [27]. Indeed, they were successful in improving bone growth at the PEEK surface.

Improving the surface bioactivity in an active manner via delivery of an osteoinductive agent is an even more challenging goal. Bone morphogenetic proteins (BMPs) have been introduced in human clinical practice in 2003 to induce bone formation by recruiting stem cells [28]. In particular, bone morphogenetic protein 2 (BMP-2) has been widely studied [14,29] and is currently used in clinical devices such as collagen sponges or pastes due to its high osteoinductivity. Recently, several concerns have been raised regarding the occurrence of adverse effects of BMP-2 such as ectopic ossification, inflammatory reaction and pain [30]. The main reason for these effects is the use of a supraphysiological dose of BMP-2 (e.g. several milligrams). There is a need to engineer new delivery systems for BMP-2 [14,29]. Coating the surface of implants was thus considered in order to localize the protein at the material surface [31].

Unfortunately, both TA6V and PEEK have a low affinity for BMP-2 and direct grafting or adsorption of BMP-2 at the implant surface leads to very low delivery of the protein or protein denaturation. In order to increase the affinity between BMP-2 and TA6V surfaces, biomimetic coatings made of HAP [32] or of biopolymers [33,34] were developed. To date strategies to deliver BMPs from the PEEK surface are still rare. Recently, Koh and coworkers [35] created a nanoporous TiO_2 surface coating to immobilize BMP-2 at the PEEK surface in the nanopores.

These formulations are promising because of the similarity of HAP and biopolymers with bone tissue constituents. The underlying strategy is that a biomimetic or a nanoporous matrix can trap, retain, and deliver BMPs locally in a more efficient manner.

The layer-by-layer technique appears to be an alternative strategy because it allows to build thin polyelectrolyte multilayer films (PEM) on any kind of material substrate with precise control of various parameters such as film chemical composition, architecture, thickness (from few nm to several μm) [36]. BMP-2 can be alternately assembled with hydrolytically degradable synthetic polymers to form a degradable PEM film releasing tunable doses of BMP-2 [37]. Recently, we have developed an osteoinductive PEM film coating made by assembly of poly(L-lysine) (PLL) and hyaluronan (HA), which is post-loaded with BMP-2 [38]. BMP-2 is trapped in the micrometer thick film and is locally delivered to cells [38]. Our preliminary studies showed that the PEM films deposited on tricalcium phosphate/HAP granules and on TA6V cylinders induced bone formation *in vivo* in a rat ectopic model [39,40]. Furthermore, the film coating can be sterilized using gamma-irradiation and the shelf-life stability of BMP-2-containing films is preserved for at least one year [40].

The objective of the present study was to assess the osseointegration of BMP-2-coated TA6V and PEEK screws implanted in rabbit femoral condyles compared to uncoated – bare – implants. Since screw implantation is a common implant model to assess implant osseointegration [12,41], we custom-designed the screws for rabbits, based on commercially available dental screws, then coated them with the BMP-2-loaded PEM films and implanted them for 4 and 8 weeks in the rabbit femoral condyle. The bone-to-implant contact and bone volume around the TA6V and PEEK screws were quantified using both histomorphometry and micro-computed tomography (μCT) analyses.

2. Materials and methods

2.1. Implant preparation

Titanium alloy (TA6V ELI, Extra Low Interstitials for clinical grade, according to norm ISO 5832-3 from HEPTAL, Neuilly-sur-Seine, France) and PEEK (TECAPEEK classix, medical implantable grade, Ensiger, Beynox France) implants were custom-made by PorteVet (France). They were specially designed for this study based on existing dental implants for clinical use in humans (Fig. 1A and B). Screw shaped implants with an upper diameter of 3.8 mm and a length of 7 mm were machined (PorteVet, France). A central hole was drilled along the vertical axis of the implant, below the hexagonal hollow cavity, to allow the screwing. Furthermore, two transversal holes were drilled along the width to assess bone formation inside the implant. These axial and transversal holes were 800 μm in diameter. All implants were cleaned using ethanol and sterilized by steam autoclave. For bare implants, no additional process was applied. For polyelectrolyte multilayer film-coated implants, after the film-coating and BMP-2 loading (see part 2.2), the film-coated implants were additionally sterilized by UV-irradiation under a cell culture hood for 20 min.

2.2. Polyelectrolyte multilayer film deposition on TA6V and PEEK implants

PEM film deposition was performed using polyethyleneimine (PEI, Sigma, France) at 2 mg/mL, poly(L-lysine) hydrobromide (PLL, 55 kDa Sigma, France) at 0.5 mg/mL, and hyaluronic acid (HA 360 kDa, Lifecore, USA) at 1 mg/mL dissolved in a buffered saline solution (0.15 M NaCl, 20 mM HEPES pH 7.4, called hereafter HEPES-NaCl buffer). The $(PLL/HA)_{24}$ films (i.e. made of 24 pairs of layer, each pair containing a PLL layer and a HA layer) were built up using an automatic dipping machine (Dipping Robot DR3, Kierstein GmbH, Germany) [40]. One layer pair of (PEI/HA) was used to enhance the PEM film adhesion onto the implant surface and the subsequent cross-linking was performed. The film-coated implants were incubated overnight at 4 °C in a cross-linking solution containing 10 mg/mL of carbodiimide hydrochloride (EDC, Sigma, France) and 11 mg/mL of N-hydroxysulfosuccinimide (sulfo-NHS, Chemrio, China) in a 0.15 M NaCl solution of pH 5.5 [38,39]. After crosslinking, the implants were roughly rinsed with HEPES-NaCl buffer and sterilized under UV.

The BMP-2 was loaded in the $(PLL/HA)_{24}$ films as previously described [39], the coated implants being incubated with the BMP-2 solution at 100 μg/mL for 90 min at 37 °C. The amount of BMP-2 adsorbed in the film and then released from it after immersion in the HEPES-NaCl buffer was quantified by fluorescence

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