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ScienceDirect

Journal of Materiomics 1 (2015) 253−261

www.journals.elsevier.com/journal-of-materiomics/

Biomineralization stimulated peri-titanium implants prepared by selective laser melting

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Received 29 May 2015; revised 11 July 2015; accepted 14 July 2015

Available online 22 July 2015

Abstract

Titanium implants prepared by selective laser melting (SLM), a method of additive manufacturing, were subjected to implantation in beagle dogs for two and four weeks. Argon ion beam-polished cross sections of the implants after *in vivo* tests were characterized by scanning electron microscope (SEM) to evaluate the bone−implant interface and the early peri-implant biomineralization with sufficiently improved resolution. Two bone mineralization mechanisms were disclosed. As early as two weeks after implantation, a layer of new bone was found to form directly on the implant surface and bone in-growth was also observed. Osseointegration was found to establish partly at the tip of the implants. After healing for four weeks it was found that osseointegration was established around the entire tip of the implants, whereas only partly at the third thread region of the implants. The experimental evidences observed reveal that an inherent highly porous surface of the titanium implants generated by selective laser melting is favorable for new bone apposition.

Keywords: Biomineralization; Selective laser melting (SLM); Bone−implant interface; Osseointegration

1. Introduction

Successful clinical use of endosseous implants is driving the need for continuing refinements in implant design and optimization of the biological healing responses following implant placement. The increasing application of dental implants led to the coining of the term of "osseointegration", defined as the direct contact between living bone and a load-carrying implant [1,2]. While this term has found considerable use in the clinical community as a means of describing the functional stability of an endosseous implant, it is a term that has eluded satisfactory scientific definition and provided little insight into the mechanisms of bony healing around implants [3,4]. Nevertheless, a careful understanding of the sequence of osseointegration in the vicinity of the interface between dental implants and hard tissues appears to be critical for enhancing early endosseous peri-implant healing. Due to technical limitations in specimen preparation, the nature of the interface between the hard tissue and the implant is hard to be evaluated. Artefacts arising from mechanical polishing of the interface often limit the resolution and prevent a more detailed, quantitative analysis of the interaction between the implant surface and hard tissue. Besides, the surface microstructure of dental implants has always been an important feature for consideration. It has been demonstrated that topographically complex implants surfaces would accelerate osteoconduction [5], which, however, is difficult to be evaluated solely by the established approach of histological study.

Selective laser melting (SLM) is a method belonging to a family of additive manufacturing technologies. It enables the direct preparation of customized components with defined

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Peer review under responsibility of The Chinese Ceramic Society.

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http://dx.doi.org/10.1016/j.jmat.2015.07.008

structures and geometries based on virtual 3D model data. Implants with controlled complex geometry and external surface morphology have been produced in one single processing step by this principle [6]. The SLM process uses powder granules of fewer tens micrometers as precursors and fuses them together via melting or partial melting. A unique rough surface is therefore formed, on which the initial powder granules stick unavoidably. The highly porous microtopography of SLM implant also greatly increases the available surface area for better adsorption of biomolecules from biological fluids. This process may offer more chance for the formation of blood clot and attachment of fibrin to the implant surface, both of which are essential to the initial bone healing process [7,8]. Recently, argon ion beam cross section polishing (CP) technique has been applied to prepare well-polished sections with negligible mechanical damage suitable for evaluating the interfacial microstructure between newly formed bone and dental implants by high resolution scanning electron microscope (SEM) [9,10].

In the present study this technique was applied to investigate the interfacial microstructure between bone formed by biomineralization and titanium implants prepared by SLM process. The specimens were collected from *in vivo* test carried out by placing SLM implants in beagle dogs for two and four weeks, respectively. The time dependent coherence and quality of the newly formed bone characterized with high resolution revealed more insights into the biomineralization and early bone formation peri-titanium implants.

2. Materials and methods

2.1. Specimens after in vivo test

Two beagle dogs of approximately 1 year of age, weighing 10 and 13 kg respectively, were used in the experiment. The study was approved by the Animal Ethic Committee, Peking University Health Science Center, Beijing, China. The dogs were in good general health, with no systemic involvement. All surgical procedures for *in vivo* test were performed under aseptic conditions by the same surgical team. During the surgeries, the dogs were pre-medicated with acepromazine (0.05 mg/kg intramuscularly) before administrating propofol (2 mg/kg intravenously). The oral mucosa and dentition were cleaned with chlorhexidine digluconate for 30 s. Local anesthesia was administered (2% lidocaine and 1:100,000 epinephrine) at the surgical sites to reduce bleeding. Three premolars (P2, P3 and P4) and the first molar of the mandible were carefully removed of each dog. The teeth were sectioned in a buccal–lingual direction at the bifurcation using a high speed fissure bur so that the roots could be individually extracted using elevators and forceps, without damaging the bony walls. Wounds were closed with resorbable sutures (vicryl 4/0 sutures). The animals received amoxicillin (500 mg, twice daily) orally during the first week after extraction and were kept on a soft diet throughout the experiment.

Three months after extraction, a mid crestal incision was made on the healed alveolar ridge to expose the bone and a full-thickness mucoperiosteal flap was reflected. A total of 12 SLM implants (TixOs® Cylindrical, Leader-Novaxa, Milan, Italy; 3.3 × 10 mm, internal hex) were inserted in dogs. The implants are commercial available products with a rough and porous surface produced by selective laser melting approach.

During the first week after implantation, the dogs received amoxicillin (500 mg, twice daily) and ibuprofen (600 mg, three times a day) orally. The dogs were fed a soft diet in order not to overload the implants. Healing was evaluated weekly and plaque control was maintained by flushing the oral cavity with chlorhexidine digluconate.

After two and four weeks of healing, the dogs were euthanized with an overdose of sodium pentobarbital. The jaws were dissected and blocks containing the experimental specimens were obtained. Subsequently, the harvested tissue blocks were fixed in a 10% formalin for 7 days.

2.2. Histological observations and SEM characterizations

After fixation and further dehydration, a total of 12 specimens, each containing one implant, were infiltrated with methacrylate, polymerized and sectioned at the buccal–lingual plane from the center of the implant using a diamond saw.

For each half of the specimen, a 200 μm thick section of the implant and surrounding tissues was cut with a diamond saw and mechanically polished using 1200 and 4000 grit silicon carbide papers until a sample thickness of 70 μm was obtained. These sections were stained with toluidine blue and were examined using the Olympus® BX51 microscope (Olympus, Tokyo, Japan). Digital images were obtained using the Olympus® DP2BSW digital camera (Olympus) connected to the microscope.

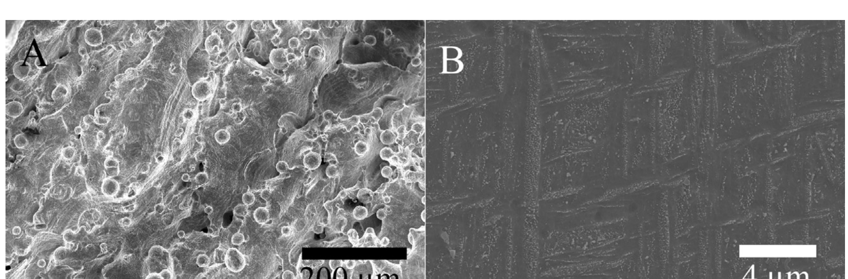

Fig. 1. SEM topographic view of the surface (A) and microstructure of a polished cross section (B) of the SLM implant.