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In vivo evaluation of plasma-sprayed wollastonite coating

Weichang Xue*, Xuanyong Liu, XueBin Zheng, Chuanxian Ding

Plasma Spray Laboratory, Shanghai Institute of Ceramics, Chinese Academy of Science, 1295 Dingxi Road, Shanghai 200050, People's Republic of China

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Abstract

Wollastonite coatings were prepared by plasma spraying. The bioactivity of wollastonite coatings was investigated in vivo by implanting in dog's muscle, cortical bone and marrow, respectively. The behaviour of bone tissue around wollastonite coatings were examined by histological and SEM observation. After 1 month in the muscle, a bone-like apatite layer was found to form on the surface of the wollastonite coating. When implanted in cortical bone, histological observation demonstrated that bone tissue could extend and grow along the surface of the wollastonite coating. The coating bonded directly to the bone without any fibrous tissue, indicating good biocompatibility and bone conductivity. SEM and EDS analysis revealed that bone did not bond to wollastonite coating directly, but through a Ca/P layer. This suggested that the formation of bone-like apatite layer was very important for bonding to the bone tissue. The amount of bone–implant contact was also measured. Wollastonite coating was shown to stimulate more bone formation on its surface than titanium coating after implantation for 1 month, enhancing the short-term osseointegration properties of implant. The test in marrow indicated that wollastonite coatings could induce new bone formation on their surface showing good bone inductivity property.

Keywords: Wollastonite coating; Plasma spraying; In vivo; Apatite; Bone

1. Introduction

Since the discovery of bioglass by Hench et al. [1] in 1969, several glasses and glass-ceramics were found to bond to living bone [2–6]. Recently, wollastonite ($CaSiO_3$) has also been regarded as a candidate for artificial bone. In vitro experiments showed that an apatite layer was formed on the wollastonite surface in simulated body fluid (SBF) [7,8]. Some reports also indicated that the formation rate of hydroxyapatite (HA) on the surface of wollastonite was faster than on the other biocompatible glasses and glass-ceramics in SBF solution [9,10]. To improve the mechanical properties, especially fracture toughness of the implants, in our previous studies the wollastonite coating was deposited on a metal substrate by plasma spraying [11,12]. The plasma-sprayed wollastonite coating showed excellent bioactivity in vitro and good mechanical properties, indicating that a wollastonite coating may be suitable for the repair and replacement of living bone, especially for load-bearing situations.

However, the environment in vivo is significantly different from in vitro. Development of bone–implant interface is complex and involves numerous factors. These include not only material-related factors, but mechanical loading, surgical technique, and patient variables such as bone quantity and quality as well. Therefore, an interest has been taken in the bioactivity of wollastonite coatings in vivo. The object of this work is to investigate the behaviour of the bone tissue around wollastonite coatings by implanting in dog's muscle, cortical bone and marrow.

*Corresponding author. Tel.: +86 21 52412990; fax: +86 21 52413903.
E-mail address: xueweichang@163.com (W. Xue).

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2. Materials and methods

2.1. Specimen preparation and characterization

Ti–6Al–4 V rods 4.0 mm in diameter used as substrate were grit-blasted and cleaned with acetone before plasma spraying. Specimens were coated with titanium and used as a bond layer by a vacuum plasma spraying system (Sulzer Metco AG, Switzerland). After that, wollastonite coatings were deposited using an atmospheric plasma spray system (Sulzer Metco AG, Switzerland). The spray parameters are listed elsewhere [13]. The thickness of coatings ranged from 200 to 300 μm.

2.2. Surgical procedure

Implants coated with wollastonite were sterilized before implantation. A total of six adult dogs weighing 12–15 kg were used. The dogs were anesthetized using a 25 mg/kg intravenous injection of pentobarbital sodium solution. Local anesthesia was given by subcutaneous injection of lidocaine solution. A straight incision was made in the area of the dog's femur. Superficial fascia and periosteal membrane were incised using a sharp dissection to expose bone surface. The implantation sites were prepared using a surgical electronic drill. The holes were gradually widened until the final size to harbour the implant. During drilling, the process was continuously cooled with saline. Before the insertion of the implants, the hole was irrigated with saline to remove the shards of bone. Wollastonite-coated implants were inserted into the holes as tightly as possible using finger pressure, followed by closing the skin with nylon suture. Four implants were inserted into each femur. Another four implants were implanted in the dog's dorsal muscles. Plasma-sprayed titanium-coated implants were also implanted as control. The dogs were sacrificed after the implantation of 1, 2, 3 months.

2.3. Histological examinations

The specimens implanted at different times were immediately fixed in 4% formaldehyde solution for 30 days after sacrifice. Specimens were dehydrated in graded ethanol solution (75%, 95%, 100%, 100%, increasing every 3 days) and embedded in polymethyl methacrylate resin. Then they were cut along the long axis of the implants using a diamond blade. The sections obtained were mounted in epoxy resin and were polished. A scanning electron microscope with electron probe X-ray microanalysis (EPMA-8705QH$_2$, Japan) was used to observe the cross-section morphologies of implants. Before the observation, a thin layer of carbon was deposited on the surface of specimens. For histological examination, the undecalcified sections were ground to a thickness of about 80 μm. After toludine blue staining, the sections were examined by light microscopy.

The amount of bone–implant contact (BIC) was measured by image analysis techniques. BIC levels were defined as the fraction of direct bone contact at the surface of the implant. The values were the mean of the five samples.

3. Result and discussion

Fig. 1 shows the SEM morphologies of the polished cross-section of the wollastonite coatings after implantation in muscle for 3 months. From Fig. 1, it can be seen that a Ca–P layer is formed on the surface of the coating. The EDS analysis demonstrates that this newly formed layer is not a biologically equivalent HA, but is a precursor phase with Ca/P ratio (about 1.43) similar to that of bone apatite. A silica-rich intermediate layer can be observed between apatite and the wollastonite coating, which is the result of the surface reaction of wollastonite with surrounding body fluids. The result of this study in muscle is similar to that of in vitro experiments [11,12]. The formation of apatite is suggested to be the direct result of the surface reaction of wollastonite coatings. These reactions included: (1) dissolution of wollastonite; (2) ion exchange of Ca for hydrogen; (3) formation of hydrated silica leach layer; (4) nucleation and crystallization of apatite.

The fact that the formation of bone-like apatite surface either in vitro or in muscle suggests that wollastonite coatings will also have good osseointegration when implanted in bone tissue. Fig. 2 shows the histological morphologies of the interface between wollastonite coatings and bone tissue after implantation for 1 and 3 months in cortical bone. After 1 month of implantation, it can be seen that a new bone is formed and fills up the gap between the implant and bone tissue (Fig. 2a). There is a distinct border between the newly formed bone and pre-existing bone. The newly formed bone is immature and full of osteocyte lacunae with osteocytes. Three months after implantation, the newly formed bone around the implant appears to be progressively replaced by a mature bone with a laminar arrangement, which cannot be discriminated from the pre-existing bone (Fig. 2b). The bone tissue is observed to be in contact directly with the implant. In some areas it was possible to observe the resorption area in the neighbourhood of the implant surface, indicating the remodelling process of the bone. It is important to highlight the absence of inflammatory cells or acute inflammation processes at the interface. There is also no indication of fibrous tissue development around the implant that can imply intolerance of the bone tissue towards the implant.