Implants coated with bioactive glass by CO_2-laser, an *in vivo* study

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Due to ageing of the population, the number of revision operations is expected to increase. Thus good fixation of medical implants is crucial for successful treatment. In our previous studies, a method to coat titanium implants with bioactive glass (BAG) via CO_2 laser treatment was introduced. It allows to localise the application of a bioactive coating, without heat treatment of the whole implant. In the present study, cylindrical titanium implants were used (BAG-coated, control group: NaOH-treated and grit-blasted Ti). Three implants were placed in each femoral epicondyle of six rabbits. After eight weeks the animals were sacrificed. Half of the implants were subjected to a torsional loading test. In the control groups, the failure occurred at the bone–implant interface, in the BAG group the failure occurred mainly in the reacted glass. The implants coated with BAG were integrated into host bone without a connective tissue capsule and were surrounded by significantly more bone than the control implants. The findings indicate clearly that the use of CO_2 laser radiation to create BAG coatings did not inhibit the bioactive properties of the glass in terms of osteoconduction.

1. Introduction

Metallic materials like titanium and its alloys are used widely for dental and orthopaedic implants. These materials are suitable for load-bearing applications. However, in many clinical cases a firm bond between the implant and the host tissues is required.

Lower survival rates have often been reported for dental implants placed in the maxilla than for those placed in the mandible. This has been related to the poor quality and insufficient quantity of bone [1, 2]. Lower implant survival rates have also been reported for the implants placed in grafted bone [3]. The number of implant operations is increasing gradually. Thus, the introduction of an implant, which could improve bone bonding and promote bone formation in its vicinity would improve implant survival in poor bone conditions.

The failure rate for hip and knee implants ranges between 7 and 23% [4, 5]. Due to the increasing age of the population in all western societies, the number of revision operations can be expected to increase in the near future. Good fixation of an orthopaedic implant to the surrounding bone is of critical importance for successful treatment. Thus, better bone bonding is also an important prerequisite for improved survival of orthopaedic implants.

Various Ca, P coatings have been developed in order to improve implant fixation in the surrounding bone tissue. Hydroxyapatite (HA) coatings mimic the mineralised component of natural bone and give a good surface for the attachment and proliferation of bone cells. HA coatings have been found to improve bone healing in experimental animals [6]. However, HA-coated implants are not used routinely in clinical practice [7].

The concept of bioactive glass was introduced by Hench *et al.* in the early 1970s [8]. Bioactivity is regarded as the ability of an artificial material to form a firm chemical bond with bone or other tissues [9, 10]. The bioactivity of the glass depends on its properties such as chemical composition, morphology of the reacted surface, charge, etc. [11–13]. When exposed to an aqueous solution, such as a body fluid, bioactive glass starts to release and attract ions such as calcium and phosphorus from the fluid. This leads to the formation of an amorphous HA at the surface of the glass. HA

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crystallises and becomes integrated into the host bone tissue through interaction with organic components of bone [10]. Released ions can also promote the proliferation of osteoblast-like cells and further improve new bone formation [14].

The release of ions and the formation of calcium phosphate and HA on the surface of a bioactive glass *in vitro* can be used to study the bioactive properties of the glass and predict its behaviour *in vivo* [15]. An established practice to test bioactive properties of a material *in vitro* is to immerse it in a simulated body fluid (SBF), a method introduced by Kokubo *et al*. [16].

Bioactive glasses have shown excellent osteoconductive properties in cell cultures [17] and in experimental bone-defect studies [18–21]. In the long run, the release of ions from the bulk glass leads to resorption of the glass [21]. This may be a desirable property when bioactive glass is used as a filler [18–21]. However, as bioactive glasses are brittle and they do not withstand bending and tension, it is practically impossible to use bioactive glasses, as such, for the manufacture of long-term load-bearing implants. Thus, the use of bioactive glasses as temporary coatings for medical devices could be a way to utilise their unique bioactive properties, especially at the early stages of the implantation [22]. A bioactive glass coating could speed up the fixation of the implant by promoting bone ingrowth and allowing faster mechanical interlocking of the implant in the host bone [22].

Traditional coating methods (enamelling, glazing, flame spray coating, rapid immersion coating, ablating/sputtering) have some limitations as far as bioactive glass coating on titanium substrate is concerned [23–25].

In our previous studies [26, 27], we have introduced a method to coat titanium implants with bioactive glass by application of a focused CO_2 laser beam in combination with the use of powder of bioactive glass suitable for repeated heat treatment. These kinds of new bioactive glasses have been developed at Åbo Akademi University [11, 28, 29]. The focused laser beam is scanned over the surface melting the glass, so heat treatment occurs locally and rapidly, without deep penetration into the material. As a result, the oxidation of the substrate is less compared with enamelling, glazing, etc. The coating manufactured by this method comprises separate interconnected droplets of bioactive glass that are firmly attached to the titanium. As our previous studies indicate, the CO_2 laser processing neither crystallised the glass nor led to major changes in the chemical composition of the glass [27]. The *in vitro* tests performed in SBF showed that the bioactivity of the glass coating remained similar to that of the control bioactive glass not subjected to laser treatment [27].

Likewise, one of the most attractive features of using a laser to create coatings is the possibility to localise precisely the effect of the laser to an area where the design of an implant would benefit from application of a bioactive coating, without heat treatment of the whole implant [30, 31]. This is especially beneficial when an implant is interfaced with different tissues, and the surface properties of an implant would match these different tissues. Besides, the fact that the laser treatment primarily affects the surface of the material can be utilised in coating medical devices that do not withstand high temperature treatments. CO_2 laser processing has been found to be a predictable and effective coating method [26, 27].

In the mid 1990s, a method of creating a bioactive titanium surface by chemical treatment with NaOH was introduced [32–35]. By this method a surface of titanium and its alloys can be modified to form a HA layer when subjected to body fluids, and in this way bond to living bone. However, despite being simple and effective, this method requires chemical treatment of the whole implant and involves heat treatment at high temperatures. Because of the positive results in terms of bioactivity reported from the NaOH treatment, this method was chosen as one of the two controls in this study.

The primary purpose of this research work was to determine whether titanium implants coated with bioactive glass can improve bone bonding *in vivo*. The specific aims were to study the osteoconductive and possibly osteoinductive properties of titanium implants coated with bioactive glass and, finally, to study the mechanical bone bonding strength of the implants in comparison with the control materials.

2. Materials and methods

2.1. Implants

Cylindrical press-fit c.p. titanium implants were manufactured for the study ($n = 36$). Fig. 1(a) shows the shape and dimensions of the implant. A total of 24 implants (non-coated control and BAG group) were grit-blasted (SiO_2, particle size = 0.1–0.7 mm) in order to clean and roughen the surface. Thereafter, 12 implants were coated with bioactive glass. In the control group, 12 non-grit-blasted implants were subjected to NaOH treatment.

After the sand blasting, prior to further treatments, all the implants were cleaned in an ultrasonic bath with acetone and ethanol for 5 and 5 min, respectively. The non-coated control implants were ready for implantation at this stage.

2.2. Bioactive glass coatings

The bioactive glass coded 1-98 was manufactured at the Åbo Akademi University, Turku, Finland. The composition of the glass is presented in Table I. After the normal melting procedure, the glass was milled into powder (< 45 μm). To clean the implant surfaces prior to the coating, the implants were grit-blasted with a bioactive glass powder (particle size 45–350 μm). Thereafter, the implants were coated with the bioactive glass from a suspension of glass powder in ethanol using a dip-coating technique. The weight ratio of ethanol to glass in the suspension was 0.7. The implants were dipped into the suspension, kept motionless, and then lifted out. The process was performed by a computer-controlled dip-coating device. After drying, the layer of glass powder obtained was irradiated with a beam from a Synrad 25 W laser (Synrad Inc., 4600 Campus Place, Mukilteo, USA). The beam was focused with a 1.5 inch ZnSe lens. The position of the laser remained fixed while the implants were scanned through the focused beam using a computer-controlled stage equipped with two stepper motors that permitted rotation around and transition

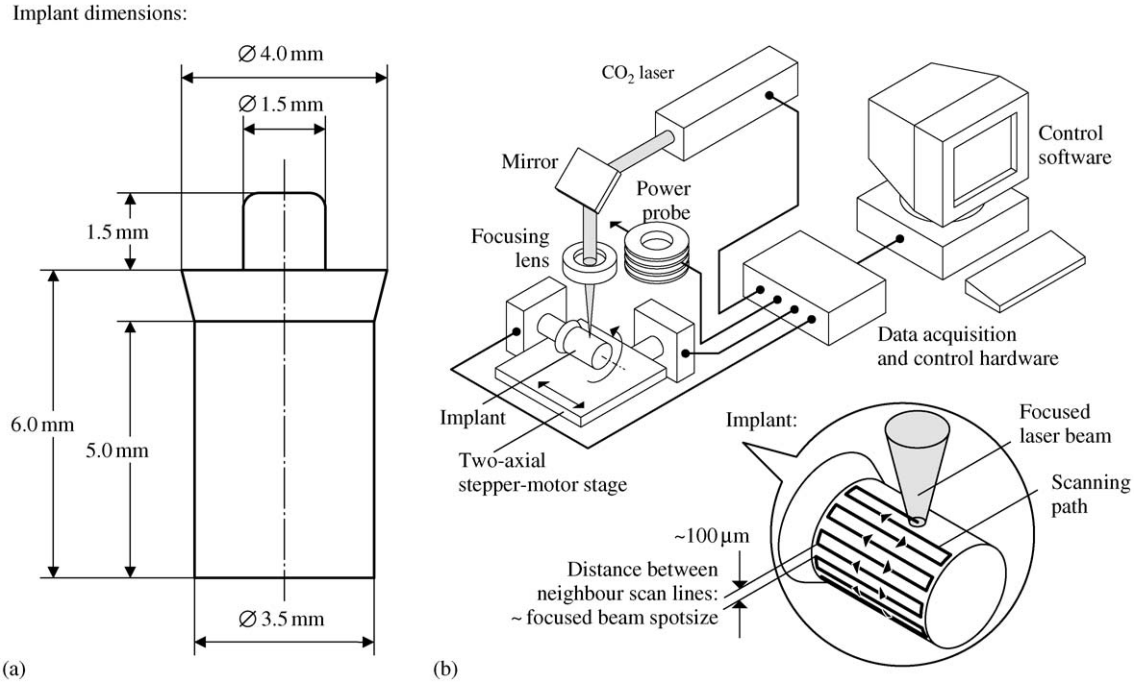

Figure 1 (a) Implant shape and dimensions. (b) Equipment used to coat implants with bioactive glass.

TABLE I Oxide compositions (expressed as wt %) of bioactive glass used in the experiment

Oxides	Bioactive glass 1-98, theoretical composition	Bioactive glass 1-98, bulk, as measured by SEM–EDX	Bioactive glass-coating created by CO_2 laser, as measured by SEM–EDX
SiO_2	53.0	55.0	55.9
Na_2O	6.0	5.1	5.7
CaO	22.0	22.5	20.5
K_2O	11.0	10.1	9.4
MgO	5.0	4.6	5.5
P_2O_5	2.0	2.4	2.4
B_2O_3	1.0	Not detected	Not detected
TiO_2	0.0	0.0	0.0

along the axis of the implant (Fig. 1(b)). The scanning with the laser beam was performed at a distance of 100 µm between the neighbouring scan lines. LabView (National Instruments, USA) software was used to create the programme that controlled both the stepper motor stage and the power of the laser.

Three-layer coatings were created on the implants. Processing was performed in air. The scanning speed of the motor stage was kept at 2.5 mm/s. The laser power was 6 W when measured after reflection from the mirror; the drop in power is approximately 10% of the output of the laser. The power of the CO_2 laser was measured using a power probe (Molectron PM5200, Molectron, Portland, USA). The equipment layout is shown in Fig. 1. The CO_2-laser irradiation melted the bioactive glass powder to numerous micro-sized ($\varnothing \sim 60$ µm) glass droplets attached to the titanium implant along the scan lines as seen in Fig. 2.

After the processing, the implants coated with bioactive glass were cleaned in an ultrasonic bath with acetone for 5 min and ethanol for 5 min.

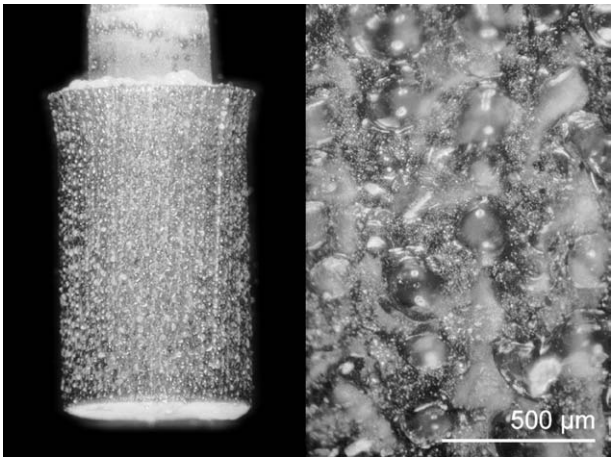

Figure 2 (a) Implant coated with bioactive glass. (b) Close-up view of the implant surface (magnification × 250).

2.3. NaOH treatment

The implants were soaked in 5.0 M NaOH aqueous solution at 60 °C for 24 h and then washed with distilled water. After drying at 40 °C for 24 h, the implants were subjected to heat treatment in a furnace. The heating rate was 5 °C/min, at 600 °C the temperature was kept constant for 1 h, after which the furnace was allowed to cool down to room temperature [33].

After the processing, the implants subjected to NaOH treatment were cleaned in an ultrasonic bath with acetone for 5 min and ethanol for 5 min.

2.4. Surgery and experimental animals

Six adult New Zealand White female rabbits (weight about 3.5 kg) were used in this study. The rabbits were anesthetised by an i.m. injection of Dormicum® (Midatsolam 1 mg/ml; Roche Oy, Espoo, Finland),

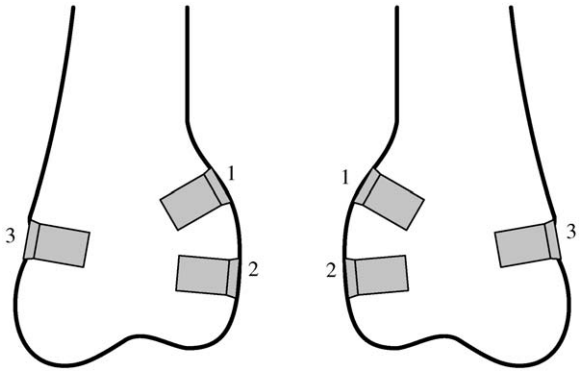

Figure 3 Schematic illustration of implantation design.

Domitor® 1 mg/ml (Medetomidin hydrochloride 1 mg; Orion-yhtyma Oyj, Espoo, Finland) and Ketalar® 50 mg/ml (Ketamine 50 mg; Warner Lambert Nordic AB, Solna, Sweden).

For the operation, the animals were immobilised and the operation area was shaved and disinfected with Klorhexol® 5 mg/ml (Clorhexidine digluconate 5 mg; Leiras Oy, Turku, Finland).

A 3 cm long skin incision was made on the medial and lateral side of the distal femur, and the fascia and periosteum were incised and retracted to expose the cortex of the medial and lateral epicondyle. The rabbit femur was chosen as the implantation site as it resembles the human maxilla, having a cancellous bone structure covered with a thin cortical bone. Two implant beds were prepared on the medial and one on the lateral side of the femoral epicondyle (Fig. 3). Preparation was started with a round dental burr and the implant beds were enlarged to a width of 3.5 mm using a set of burs with a gradually enlarging diameter.

All the soft and hard tissue preparations were made under aseptic conditions using low speed drilling with sterile saline irrigation (Natriumklorid Braun 9 mg/ml; B. Braun Medical Oy, Espoo, Finland).

The implants were placed according to a previously designed balanced split blot table (Table II). The implants from the right femur were subjected to a biomechanical test and later histological evaluation, while the implants from the left femur were used for histological and histomorphometric examination.

The fascia and skin were closed separately in layers with synthetic absorbable sutures (Vicryl® rapid 3-0; Ethicon GmbH & Co. KG, Norderstedt, Germany). The same surgical procedures were performed bilaterally for right and left femurs.

TABLE II Balanced split-plot design of the experimental and control implants in the study animals

	Right femur			Left femur		
Number of rabbit	1	2	3	1	2	3
1	A	B	C	B	C	A
2	C	A	B	A	B	C
3	B	C	A	C	A	B
4	B	C	A	C	A	B
5	C	A	B	A	B	C
6	A	B	C	B	C	A

A = c.p. titanium implant; B = BG-coated implant; C = NaOH treated implant.

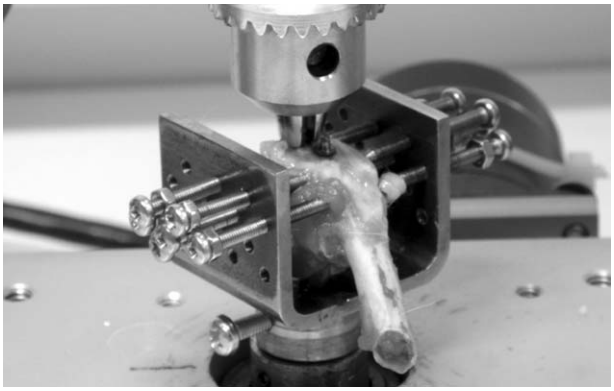

Figure 4 Biomechanical test.

The animals were given post-operatively i.m. Temgesic® 0.3 mg/ml (Buprenorphinum 0.3 mg; Schering-Plough, Brussels, Belgium) for post-operative pain.

During the study, the animals were kept in cages and fed with standard hard diet pellets with water *ad libitum*. National guidelines for experimental animals were carefully followed.

After eight weeks, the experimental animals were sacrificed with an overdose intravenous injection of Mebunat® vet. (Pentobarbital.natr. 60 mg; Orion-yhtymä, Espoo, Finland).

The femoral bone and implants were exposed *en bloc*. The bones were cut laterally beyond the site of implantation using a diamond disk. The bone blocks were stored and transferred on ice for biomechanical testing performed immediately after the samples were harvested.

2.5. Biomechanical test

The strength of the bone–implant interface was studied mechanically for one-half of the implants in each group. For the study, a torsional loading test was performed using a universal testing device (Avalon Technologies, Rochester, MI, USA). Prior to the test, the equipment was calibrated according to the operating manual.

The implant was first fixed to a drill socket permanently attached to a load cell, and then the bone was carefully mounted on a U-profile steel holder and fixed firmly with pointed screws. The holder was permanently attached to a stepper motor. Thus, the axis of the implant was aligned with the axis of the stepper motor and the load cell, as shown in Fig. 4.

A torque force was applied by rotating the stepper motor at constant speed of $0.195°s^{-1}$ until failure of the implant occurred. The force was recorded by the load cell. The data from the load cell were sampled by a computer-controlled data acquisition system at the rate of 20 Hz. The software for the test was written under Visiual Designer 3.0 (Intelligent Instrumentation, Tucson, AZ, USA).

2.6. Histological and histomorphometric analysis

All the specimens $(n = 36)$, including the mechanically tested ones, were studied by histological and histomor-

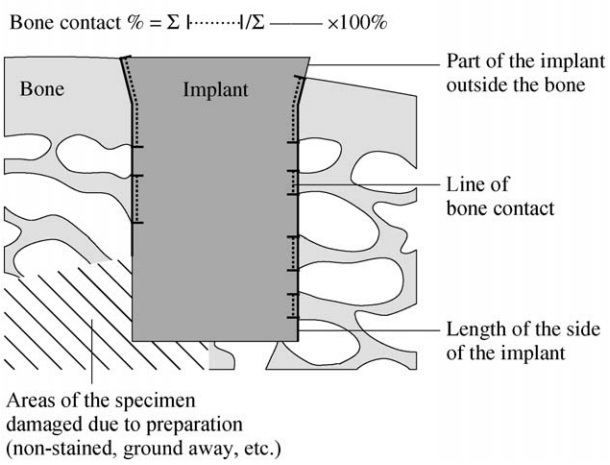

Figure 5 Histomorphometric examination of the implant–bone interface. Bone contact was calculated as the ratio of the bone in immediate contact with the implant to the total length of the sides of the implant. If a part of an implant was outside the bone, and/or an area of a specimen was damaged due to preparation, these were discarded from the calculation.

phometric analysis. For the analysis, the samples were embedded in methylmetacrylate and sectioned into two halves along the axis of the implant. From the halves, 20-μm thick undecalcified specimens were prepared. For the procedures, a standard cutting–grinding technique was used [19]. Specimens from mechanically tested implants were stained with the Mason trichrome method. The specimens from the intact implants were stained with the Van Gieson method. All the specimens were histologically analysed using a light microscope.

Computerised histomorphometry (CH) was performed on the specimens stained with the Van Gieson method using a light microscope coupled with a computerised image analysis system (MicroScale TC; Digithurst Ltd., Royston, UK) [36]. As the working size of the digitised image (24 bit, colour) was limited to 720 × 512 pixels, instead of using a still image of the whole implant and surrounding bone [19, 20], the measurements were performed in live mode, allowing the use of a high microscope magnification. This enabled interactive local evaluation of the bone–implant interface with the specimen being moved in the course of the measurement.

Bone apposition or ongrowth was measured as the sum of the fractional linear extents of bone in the immediate contact with the surface of the implant reported as a percentage ratio of bone in contact with the implant to the length of the implant side available for bone apposition [37] (Fig. 5).

As these types of measurements usually involve major interobserver variations, three different observers performed the measurement procedure independently [37]. Observers 2 and 3 had considerable experience with CH, while observer 1 performed the examination for the first time.

2.7. Scanning electron microscopy (SEM) and EDX analysis

The specimens for the SEM examination were prepared from the samples embedded in methylmetacrylate after the histological specimens were made.

The interface between the implant and the bone was examined by SEM (JEOL JSM 5500, Tokyo, Japan). In the case that the implants had been subjected to the biomechanical test, the location of the fracture was of primary importance. EDXA (hardware: Princeton Gamma-Tech, Princeton, NJ, USA; software: Spirit, Princeton Gamma-Tech, Princeton, NJ, USA) was used mainly to study the interface between the implant and the bone, as well as the compositional changes in the bioactive glass caused by the interaction with body fluids.

3. Results and discussions

3.1. Biomechanical test

The results of the mechanical test are presented in Fig. 6. To study the differences between the groups, one-way analysis of variance was performed on the data using SigmaStat 2.03 statistical software (SPSS Inc., Chicago, USA). The torsional loading test showed no significant differences in the interfacial bonding strength of the implants to the surrounding bone. This may be due to the smooth topography of the implant surface. There is no place in the implant profile for mechanical interlocking of the implant in spite of the significantly increased new bone ingrowth (histomorphometric analysis).

3.2. SEM and EDX analysis

SEM examination revealed that in the case of both control group materials, failure had occurred at bone–implant interface. The thickness of the layer at the surface of titanium created by NaOH treatment appeared to be too thin to be detected by SEM. Therefore, it is not possible to conclude whether the failure occurred at the implant-coating or coating-bone interface.

In the case of bioactive glass, the failure occurred at the bone–implant interface in the areas between the glass-coating droplets. As far as the glass droplets of the coating are concerned, the failure occurred primarily inside the glass. EDX analysis did not reveal any distinct reaction zones inside the glass droplets, as most of the glass was crushed.

EDX analysis also revealed that the bioactive glass granules comprising the coating had reacted completely and mainly consisted of silicon, calcium and phosphorus

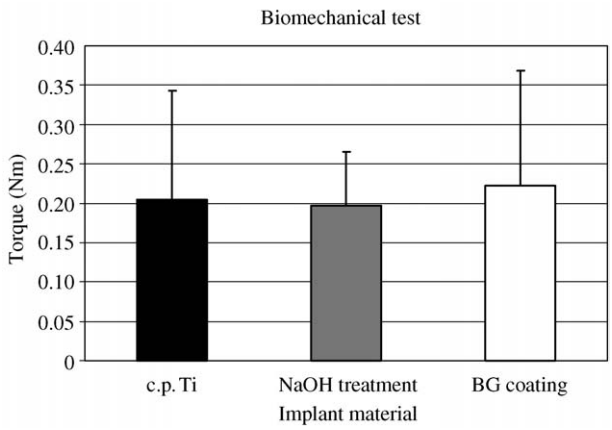

Figure 6 Results of the biomechanical test. No statistically significant differences between the groups.

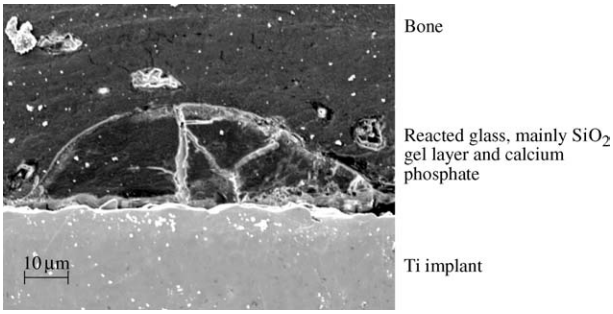

Figure 7 A SEM image of the remains of a bioactive glass droplet on the surface of the titanium implant after eight weeks of implantation. The droplet is fully incorporated in bone. The cracks are either due to the mechanical test or due to preparation of the specimen.

(Fig. 7). Thus, it is possible to assume that, in terms of the strength of attachment of the bone to the implant, it is the reacted glass that is the weak point at eight weeks after implantation.

3.3. Histological and histomorphometric analysis

Histological analysis revealed that the implants coated with bioactive glass were integrated into host bone without a connective tissue capsule. The Van Gieson staining at the implant–bone interface was less intense than that of the bone, but osteoblasts were also present at the interface layer. Thus, the bone was structurally in direct contact with the glass droplets and the surface of the implant in between the droplets. Morphologically, both newly laid woven bone and mature bone contained clearly visible osteocytes and Haversian canals. No inflammatory reaction was observed.

The implants processed with NaOH were also well integrated into the host bone without a connective tissue capsule. No inflammatory reaction was observed. The woven bone around the implant seemed less organised than in the case of implants coated with bioactive glass. The woven bone at the interface was characterised by dense lamellae with only a few embedded osteoblasts.

The titanium implants without a coating were surrounded by immature bone osteoid, woven bone, and a dense thin fibrous tissue capsule with mild to moderate inflammatory reaction. Histological images are presented in Figs. 8–11.

The results of histomorphometric examination of the interface between the bone and the implant clearly indicate more bone surrounding the implant with bioactive glass coating in comparison with both control materials. To test the differences between the groups (the three observers and the three materials) two-way analysis of variance along with Tukey tests were performed on the data using SigmaStat 2.03 statistical software (SPSS Inc., Chicago, USA). The statistical analysis showed significant difference between bioactive glass coatings and the two controls for the three observers. The difference between the control groups of the materials (c.p. titanium and NaOH treatment) was not significant (Fig. 12). There was a significant difference between the first observer (with little experience) and the other two observers. Thus, the data from the first observer were discarded and the statistical analysis performed again. The analysis

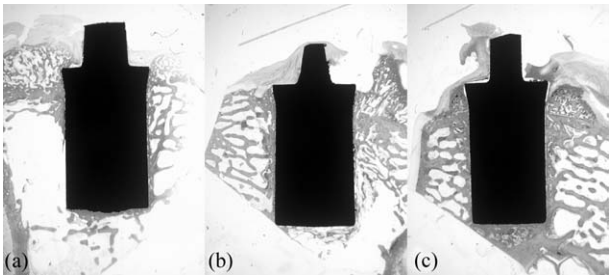

Figure 8 Histological slices of the implants (a) c.p. Ti (b) NaOH-treated Ti, and (c) BG-coated Ti.

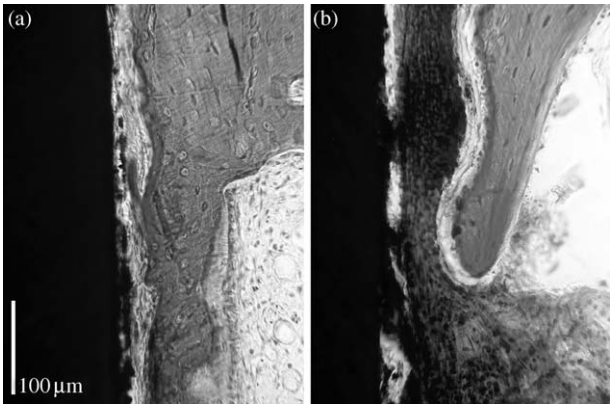

Figure 9 Interface of a c.p. Ti implant and surrounding tissues (a) fibrous capsule and (b) inflammation reaction.

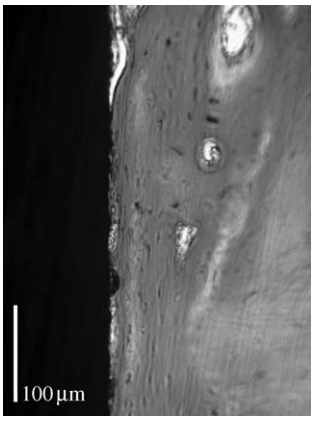

Figure 10 Interface of a NaOH-treated implant and surrounding tissues.

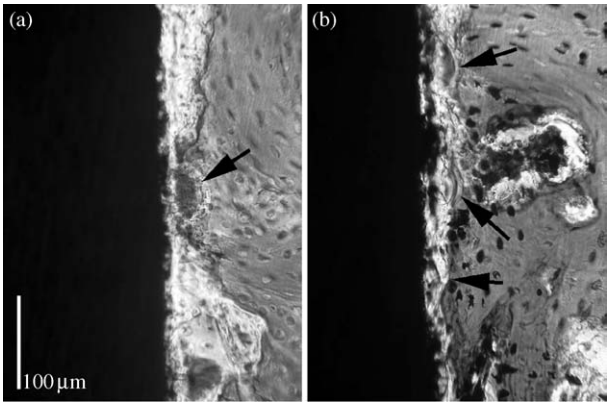

Figure 11 Interface of a bioactive glass-coated implant and surrounding tissues. Arrows point at bioactive glass droplets.

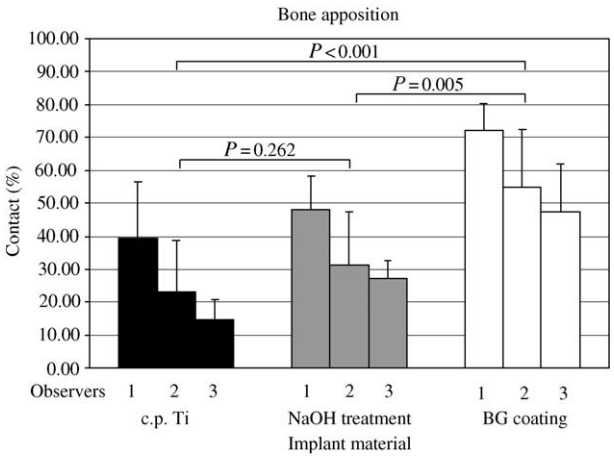

Figure 12 Results of the histological examination of bone–implant interface.

indicated a significant difference between the bioactive glass coating and both controls (c.p. titanium and NaOH treatment) with no significant difference between the observers.

4. Conclusions

The use of CO_2 laser allows selective coating of cylindrically shaped titanium implants. In terms of mechanical strength of the interface between the implant and the host bone eight weeks after implantation, no differences between the bioactive glass-coating group and both control groups were observed. In the control groups, the failure occurred at the bone–implant interface, whereas in the bioactive glass group the failure occurred mainly in the reacted glass (Si-gel). Nevertheless, in the biomechanical test, the implants coated with bioactive glass performed at least as well as both control materials in terms of interfacial bonding strength of implants to host bone.

Moreover, from the point of view of bone apposition, the implants covered with bioactive glass were surrounded by significantly more bone than the control implants. This finding clearly indicates that the use of CO_2 laser radiation to create bioactive glass coating did not inhibit the bioactive properties of the glass in terms of osteoconduction. Thus, the method may open the way to a number of novel applications of bioactive glass.

As mentioned in the introduction, bioactive glass coatings are not intended to remain permanently, but will react with body fluids and be resorbed. Therefore, they could be used as the bioactive part of a complex implant design, which would allow for a proper mechanical interlocking of the implant in the bone.

The use of CO_2 laser allows the creation of a coating for a predefined area on the implant surface. The possibility to control the transition and power of the laser beam provides the potential for processing implants with complex geometry, patterning of the surface, etc. Moreover, it allows the use of bioactive glass locally in combination with other types of coatings and/or surface treatments, realised by the same laser [38]. Thus, the surface of the same implant can be modified locally to match the needs of the different surrounding tissues [38, 39].

Acknowledgments

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