J Mater Sci: Mater Med (2009) 20:1721–1728
DOI 10.1007/s10856-009-3730-4

The early osseointegration of the laser-treated and acid-etched dental implants surface: an experimental study in rabbits

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Received: 1 December 2008 / Accepted: 3 March 2009 / Published online: 17 March 2009

Abstract The aim of the study was to evaluate early osseointegration of the laser-treated and acid-etched implant surface after the installation in rabbit tibias for 4 weeks. A total of 56 screw-shaped implants were grouped as follows: group A: implants were turned surface; group B: implants were laser-treated surface; group C: implants were acid-etched; group D: Implants were laser-treated and acid-etched surface. After 4 weeks, the removal torques were: group A: 13.21 $\pm$ 11.30 Ncm; group B: 29.73 $\pm$ 8.32 Ncm; group C: 30.31 $\pm$ 9.45 Ncm; group D: 35.76 $\pm$ 7.58 Ncm; The averages of bone-to-implant contact (BIC) were as follows: group A: 27.30 $\pm$ 6.55%; group B: 38.00 $\pm$ 8.56%; group C: 42.71 $\pm$ 8.48%; group D: 49.71 $\pm$ 9.21%. The removal torque and bone-to-implant contact measurements yielded statistically significant differences between the treated groups and turned group ($P < 0.05$); The laser-treated and acid-etched surface achieved higher Bone-to-Implant Contact than the laser-treated surface ($P < 0.05$), but there was no statistically significant difference between the laser-treated and acid-etched surface and the acid-etched surface in bone-to-implant contact ($P > 0.05$). In the present study, it was concluded that the laser-treated and acid-etched implants had good osteoconductivity and was a potential material for dental implantation.

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1 Introduction

Numerous studies have showed the effects of implant surface's chemistry and topography property on biocompatibility with bones in vivo and vitro experiments [1–3]. Surface treatments, such as surface sandblasting, acid-etching, anodic oxidation etc. can be further conducted to improve the quality and quantity of the bone-to-implant interface [4–7]. In recent years, it has been proved that using laser-etching technique to alter the topography of the implant surface can greatly improve contact between bones and implants [8]. Unlike the other surface treatments, laser-etching technique can create unique 3D geometries accurately; it is a new method to produce a high degree of purity and with enough roughness for good osseointegration [9].

Meanwhile, it suggests that primary and secondary macro and microstructures had significantly effect on osseointegration results [5]. More and more dental implants were treated to create this surface morphology, such as the Straumman implant surface (SLA, Sand-blasted Large grit Acid-etched) and Tiunite implant surface (macroscopic groove and microscopic pore). As we know, primary and secondary macro and microstructures could be made by the method of SLA (Sand-blasted Large grit Acid-etched); But the Sand-blasted method represented a stochastic surface modification, so it would jeopardize the bone quality [10]. It also had some pollution to the implant surface and couldn't make 3D geometries [6], Gold J [11] reported the positive correlation of surface roughness with bone response, which requires a surface with a controlled, non-random structure. The acid-etch method was widely used to modify the implant surface. Some studies had shown that the acid etched implant provided an excellent surface for bone-to-implant integration [7, 12, 13]. However, neither acid-etch method was able to create bigger pits, nor could it greatly increase the bone and implant's contact area.

Sung-Am Cho [14] had reported the laser treated implant surface could got higher removal torque values compared to the turned implant surface. Whether the combination of the laser-treated and the acid-etched method will benefit the bone-to-implant integration still remains unknown at present. An attempt of this ideal treatment is thus made here. The purpose of the present study was to evaluate whether this surface morphology had better osseointegration than the laser-treated surface in early period.

2 Materials and methods

2.1 Implant design and surface treatment

The pure titanium (TA_2, Baoji, Shanxi Province, China) was selected for implant material. A total of 56 screw of shaped, commercially pure titanium implants (diameter 3.4 mm, length 8 mm, Southern Medical University, China) were divided into four groups: group A (Turned implants), group B (laser-treated implants), group C (acid-etched implants), group D (laser-treated and acid-etched implants). The screws were taken directly from the sterile package, without any additional preparation prior to laser machining. The laser micro machining was carried out in atmosphere, at 1064 nm wavelength etching, at a pulse frequency of 7.8 kAZ and an energy of 180 mj/pulse using a pulsed Nd:YAG laser (LX3, Laser Company, Xingchen, Shenzhen, China). After the laser-treated, 14 implants were acid etched with the mixture of 18% HCl and 49% H_2SO_4 for 40 min at a high temperature (60°C).

2.2 Surface characterisation

2.2.1 Scanning electron microscope (SEM)

Topographic evaluation was performed with SEM (Quanta 400 FGE, Holland) to compare four different implant surfaces. The SEM parameters were: 20.0 kV electron energy, high vacuum. Each screw was analysed at 25 and 500 magnification.

2.2.2 Electron-probe microanalyzer (EPMA)

Electron-probe microanalyzer (EPMA) (JEOL, JXA-733, Japan) was used in this experiment to evaluate the element analysis of four implant surfaces. The EPMA parameters were: 20.0 kV electron energy, 400 μm beam diameter. Each screw was analysed in two spots, including several ablation pits each, on the second thread flank.

2.3 Optical Profiler

Seven randomly chosen implants from each group were analysed with Optical Profiler (NT1100, Veeco, USA), which area of measurement was 736×480 μm. The parameters were: 10.28 Magnification, 816.72 nm, Sampling. Implants were analysed before implant insertion. The surface parameters evaluated were the average height deviation value (Sa), the maximum peak-to-valley roughness (St) and the developed surface area ratio (Sdr). Each screw was analysed in two spots, including several ablation pits each, on the second thread flank. The averages of measurement data were taken.

2.4 Animals and surgical technique

Animal selection, management and surgery protocol were approved by the medical experimental animal center of Guangdong province. 14 adult white rabbits weighing 2.5–3.0 kg were used and were divided into four groups through stochastic grouping. The animals were anesthetized with Sumian Xin (Animal Husbandry Research Institute, Jilin, China. 0.15 ml/kg of body weight) intramuscularly. Before the surgery, 0.6 ml Primacaine (Merignac Cedex, France, 0.2 ml/kg of body weight) was injected locally into the surgical sites of tibia metaphysics. Four different implants were placed in two sides tibia Stochastically, which were about 7–12 mm below the joints. After the surgery, all the animals were injected Benzylpenicillin (Pharmaceutical Company, Sichuan, China) at a dose of 0.3 mg per animal. They were allowed full weight bearing and movement after surgery. Four weeks later, the animals were sacrificed using an overdose of anesthetics.

2.5 Removal torque measurements

Four weeks post operation, 7 rabbits with a total of 28 implants were sacrificed. The implant sites were surgically exposed, the bone and soft tissues that had formed on top of the implants were carefully removed. Subsequently, the force needed to unscrew the implants ($n = 28$) was measured using a digital torque gauge (Mark-10 corporation, USA). The result was recorded by measuring the maximum removal torque between implant and bone where fracture occurred.

2.6 Specimen preparation for histologic evaluation

Four weeks post operation, the other 7 rabbits with a total of 28 implants were sacrificed for histological evaluation. Tibias and the implants with the surrounding bone were removed, cleaned of soft tissue, and fixed in 10% buffered formalin. Four groups implant specimens

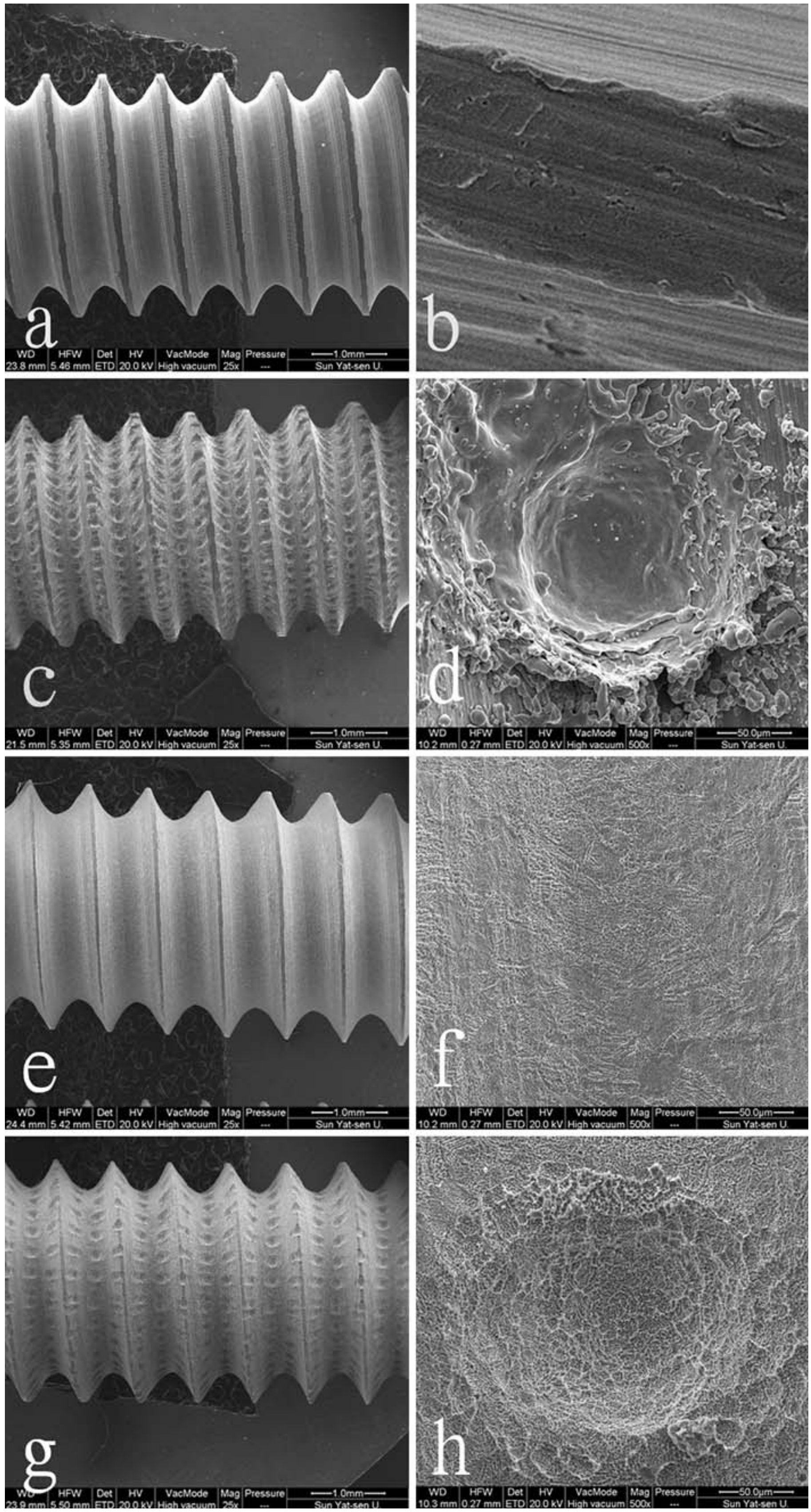

Fig. 1 SEM-analysis of four different implant surfaces topographic: **a**, **b** turned surface; **c**, **d** laser-treated surface; **e**, **f** acid-etched surface; **g**, **h** laser-treated and acid-etched surface (magnification 25× and 500×)

were dehydrated using a series of ascending alcohol water solutions, ending with 100% resin and embedded in methyl-methacrylate. They were cut in a bucco-lingual direction and parallel to the axis of the implants by a low-speed diamond saw with coolant (Lecia SP1600, Lecia Company, German), 150–200 μm thickness per implant section, grinding were performed as described by Wang Dongsheng [15] until the specimen thickness reaches 60 –80 μm. They were stained using methylene blue-acid fuchsin. These were evaluated under Optical microscope (Olympus BX41, Olympus Co., Japan) and image analysis software (Image-pro Express 6.0, Media Cybernetics Inc., USA) to allow a quantitative measure of the bone-to-implant contact (BIC). Each implant was repeated three times, the averages of measurement data were taken.

2.7 Statistics

Data from the topographical evaluations were analysed using the Games-Howell test. Data from the histomorphometrical were analysed using the non-parametric Kruskal–Wallis test. All statistical testing were carried out at the 5% significance level. Box plots are given to describe the response variables in a non-parametric manner. The data are presented as a mean value with the standard deviation. The differences among 4 groups were evaluated by the SPSS 13.0 program (SPSS, Inc, Chicago, IL, USA).

3 Results

3.1 Topographic evaluation

The surface morphology of the specimens was observed through scanning electron microscopy (SEM) (Fig. 1): group A reveals the machining lines and relatively smooth surface; Group B had many deep pits with a size of 100 μm, the ablated material was melted, forming a ridge around the pits; Group C yields a considerable surface roughness with different sizes of micro pore of 1–3 μm; Group D showed significant surface roughness with macro pits of 100 μm and micro pore of 1–3 μm of different sizes, a ridge around the pit disappeared.

3.2 Electron-probe microanalyzer (EPMA)

The few carbon and oxygen elements were examined by electron-probe microanalyzer (EPMA) on group A surface. Other three groups showing clean surface with only Ti-peaks (Fig. 2).

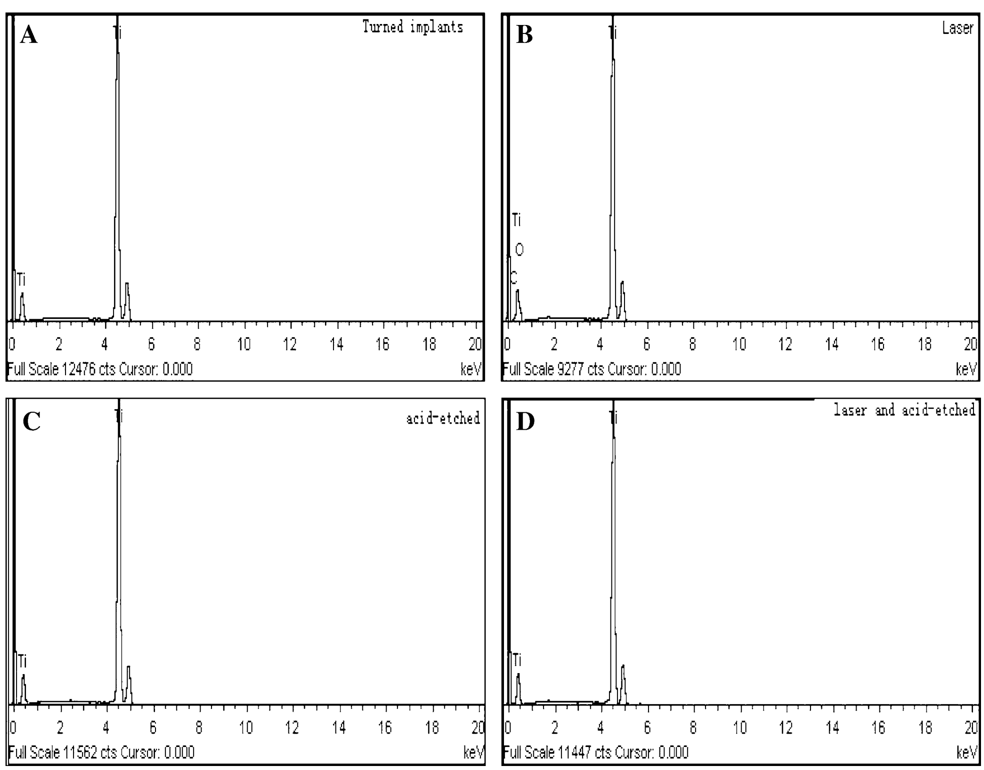

Fig. 2 EPMA-analysis of groups **a**–**d**. **a** turned surface; **b** laser-treated surface; **c** acid-etched surface; **d** laser-treated and acid-etched surface

Table 1 Surface parameters of the used implants ($n = 7$)

Group	Surface treatment	Sa (μm)	St (μm)	Sdr (μm)
A	Turned	0.16 ± 0.29	6.47 ± 1.15	0.21 ± 0.04
B	Laser-treated	10.26 ± 2.19	78.65 ± 12.02	14.75 ± 2.27
C	Acid-etched	0.82 ± 0.09	11.86 ± 0.54	1.03 ± 0.11
D	Laser-treated and acid-etched	7.49 ± 1.22	85.92 ± 8.75	11.83 ± 1.26

Sa arithmetic mean deviation of the surface, *St* maximum peak to valley height of the surface, *Sdr* developed surface area ratio

3.3 Optical Profiler

Surface roughness analyses revealed four different surfaces. The value of Sa, St and the Sdr were summarized in Table 1. The test surfaces had a significantly higher Sa, Sdr and St value than the surface of the turned implants ($P < 0.01$). The values of Sa, St and Sdr of groups B and group D were significantly higher than the group C; But the Surface parameters between groups B and group D were not statistically significant ($P > 0.05$).

3.4 Removal torque measurements

Four weeks after implants placement, the Mean values of the removal torque was 13.21 ± 11.30 Ncm for the turned implants, 29.73 ± 8.32 Ncm for the laser-treated implants, 30.31 ± 9.45 Ncm for the acid-etched implants, 35.76 ± 7.58 Ncm for the laser-treated and acid-etched implants. The torque measurements yielded statistically significant differences between the test groups and the turned group ($P < 0.01$). However, the data deviation among the test groups were not statistically significant ($P > 0.05$). The removal torque results were summarized in Table 2 and were described in Fig 3a.

3.5 Histological observations/histomorphometrical analysis

The mean values and percentages of BIC% for each group at 4 weeks were presented in Table 3. In general, no implants were lost over the study period. All implants

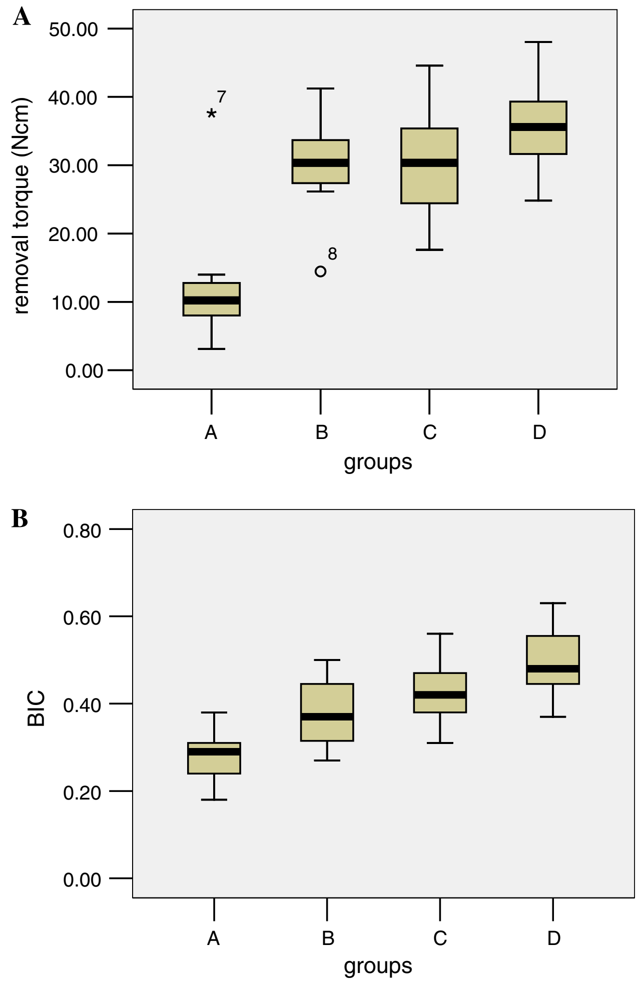

Fig. 3 **a** Comparisons of Mean values of the removal torque in each group. **b** Comparisons of the bone-to-implant contact (BIC) in all threads of groups A, B, C and D after 4 weeks of healing period

histologically demonstrated newly formed bone into the peri-implant bone chambers, but the accuracy of the implant channels markedly differed among four groups, especially in the apical area of the implant supporting bone. Only few new woven bone was found on the surface of group A (Bone-to-Implant Contact: 27.30 ± 6.55%). Test groups were better than turned group ($P < 0.01$); More newly formed bone was observed in close contact with the implant surface of group D (Bone-to-Implant Contact:

Table 2 Mean values of the removal torque in each group (Unit: Ncm) ($n = 7$)

Group	Surface treatment	Mean values	Std. deviation
A	Turned	13.21	11.30
B	Laser-treated	29.73	8.32
C	Acid-etched	30.31	9.45
D	Laser-treated and acid-etched	35.76	7.58

Table 3 The mean percentages of the bone to implant contact (BIC) performed in all threads ($n = 7$)

Group	Surface treatment	BIC (%)	Std. deviation
A	Turned	27.30	6.55
B	Laser-treated	38.00	8.56
C	Acid-etched	42.71	8.48
D	Laser-treated and acid-etched	49.71	9.12

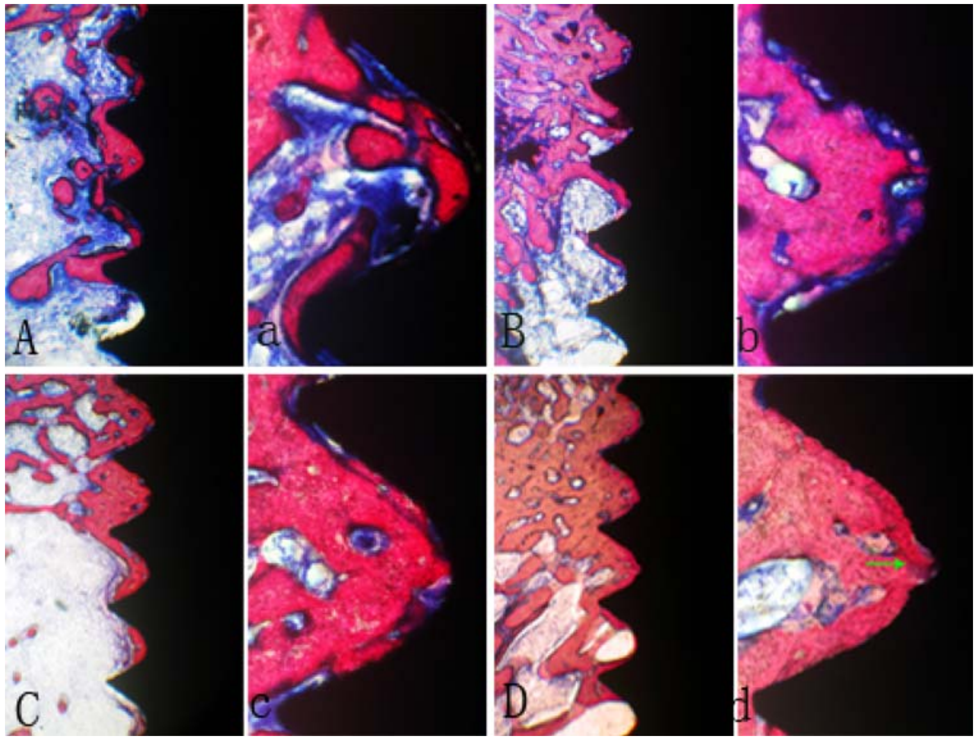

Fig. 4 Histological views (methylene blue-acid fuchsin, **A**–**D** original magnification 40×; **a**–**d** magnification 100×) of the implant and peri-implant tissues 4 weeks after different implants placement. Few newly formed woven bone was observed in contact with the control group surface (**A**, **a**); The higher degree of bone was present in surface of group B compared to group A (**B**, **b**); Newly formed woven bone was present at the bone-implant interface (**C**, **c**); Newly trabeculae and rather mature bone was observed in close contact with the implant surface (**D**), some new bone was observed growing into the pits (**d**, *arrows*)

49.71 ± 9.12%) than the implant surface of group B (Bone-to-Implant Contact: 38.00 ± 8.56%) ($P < 0.05$). The volume density of the scaffold of group D had increased both in the formation of new trabeculae and in deposition of more mature ones, the newly formed bone was observed for the pits of implant surface (arrows) (Figs. 3b and 4); But there was no statistically significant differences between group C and group D, also between group B and group C ($P > 0.05$).

4 Discussion

Laser-etching technique has been demonstrated to be a cleaner and easier method for the implant surface modification method recently [16]. Ari I et al. [17] reported that the pore of 100 μm was large enough for the consistent growth of new bone within the porous space, it won't reduce the mechanical strength of the implant. In present study, in order to achieve higher mechanical interlocking of bone-to-implant interface, the laser-etching technique was used to create macro pits of 100 μm to increase surface area greatly, and then the acid-etched method was used to improve bone conduction of implant surface. We found that consistent pits were made accurately by the laser-etched on the screw implant surface, the primary and secondary macro and microstructures on implant surface could be obtained by the laser-treated and acid-etched method at certain parameters. By changing the laser parameters, different sizes of pits were easy access. The elements of carbon and oxygen were examined after the laser-treatment in present study. Carin Hallgren et al. reported [8] that possible reasons for this could be residue from the cleaning procedure with organic solvents and surfactants or from the ambient atmosphere. At present, it remains uncertain whether these two elements are conducive toosseointegr-ation, but an evaluation of the histological sections revealed that laser-treated titanium implant had a higher percentage of BIC than the turned surface. The further researches were still needed.

Numerous research groups supported the development of rough implant surfaces of various topography which positively affects the cell migration, adhesion and increased bone apposition [1–3, 18]. A research for the optimal surface roughness has still an ambiguous answer. Wennerberg A [19] reported that screw-shaped implants with an average surface roughness of about 1.5 μm were found to be optimal for bone growth, based on removal torque tests experiment; But H.J. Ronold [20] found that an optimal surface roughness of coin-shaped implants for bone attachment was in the range of 3.62–3.90 μm, which surface was blasted with TiO_2 particles and etched hot hydrochloric acid. When the Sa was above 3.90 μm, a negative correlation between Sa, St, and Sdr values and bone fixation. In present study, the surface roughnesses of the laser-etched surface and laser-treated and the acid-etched surface were above 3.90 μm, but favorable osseointegration also achieved. The optimal surface roughness the laser-treated still remains unknown at the moment. Maybe different surface treatment has its optimal surface roughness. Meanwhile, we found that the laser-treated and acid-etched surface had higher BIC than the laser-etched surface, but there were no statistically significant differences in the removal torque values among three test groups. Although no statistically significant difference in removal torque values, the laser-treated and acid-etched surface had a slightly better performance when compared to the acid-etched surface in optical microscope.

In addition, some authors suggested that the BIC of different implant surfaces exists no statistically significance after 8 weeks. Chang et al. [21] demonstrated the RGD-modified SLA surfaces demonstrated nearly the same amount of bone apposition as the SLA control implants after 4 weeks; Niklaus P, Lang [22] reported that the SLA surface implant had no statistically significant compared to the machined surface after 6 weeks. These suggested that period of 2–4 weeks was fit for observing an early osseointegration in implant surface treatment study. So 4 weeks period was chosen in our study. After 4 weeks, a significant difference existed between the test groups and the turned group in BIC and the removal torque values. However, 8–12 weeks were usually selected as the observation periods in removal torque of different implants by numerous research groups [14, 20, 23]. In our study, no statistically significant differences existed among test groups in removal torque values. Maybe 4 weeks time was too short and the implant sample size was small for comparing the removal torque of different surface implants. Different periods and greater implant sample size will be required in next animal experiments.

As we know, the study of mechanism of osseointegration is rather complex. The experimental result suggested this idea was feasible for titanium implant surface modification. The laser-treated and acid-etched surface had been confirmed with better osseointegration than the laser-treated surface. Further researches in laser-treated and acid-etched surface were still needed.

5 Conclusions

To summarize, the method of laser-treated and acid-etched was feasible for titanium implant surface modification. The observed significant enhancement of new bone apposition to the laser-treated and acid-etched surface during the early stages of bone regeneration. It had better bone conduction than the laser-treated surface and was a potential material for dental implantation.

Acknowledgements Project name: (1) National Science/Technology Pillar Program in the Eleventh Five-year Plan Period. Serial number: 2007BAI18BO6; (2) Science and Technology Three Item of Funds Plan Project of Guangdong Province. Serial number: 2006B19901006. This work was supported by the Guangdong Provincial Stomatological Hospital (Southern Medical University, China) and Guangdong Medical College.

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