In the process of peri-implant endosseous bone healing, the first and most important stage is osteoconduction. With the formation of peri-implant blood clots, a series of cytokines and growth factors are released by the activated platelets, consequently stimulating the recruitment and migration of osteogenic cells to the implant surface. Ions conducive to bone formation, such as strontium and calcium-phosphorus, can be added to the surface by chemical modification. These ions could promote platelet activation and strengthen fibrin binding to the implant surface. In addition, surface morphology is vital to osteoconduction. With the increased surface area, microscale morphology may lead to a promotion in fibrinogen absorption. It affects not only the amount and extent of platelet activation but also the anchoring function of the temporal scaffold through which cells attach to the surface of the implant. In the second healing stage, new bone formation begins, which results in a mineralized interfacial matrix at the healing site. The third healing stage is bone remodeling.

During the past few years, implant surface treatment techniques have been intensively studied and improved, including sandblasting with large grit and acid etching (SLA), resorbable blasting media (RBM), and anodization. At present, SLA is generally accepted as the most extensively used and basic technique for implant surface treatment confirmed by in vitro and in vivo researchers. Studies have proven that changes of surface roughness by SLA can influence selective protein adhesion, collagen synthesis, and osteoblast proliferation directly. Increasing surface roughness can promote osteoconduction; that is why...
most commercial implants have a microrough surface. In addition, the surface treatment technique of RBM involves carving microtopography on titanium (Ti) implants with microparticles of calcium phosphate (CaP) and acid etching. A CaP-containing external layer on the implant surface by RBM is suggested to improve osseointegration.14–17 Furthermore, unremovable microstructure and nanostructure can be formed on the surface of biomaterials by acid etching, plasma spray, hydrothermal treatment, alkali treatment, or a combination of various methods.18 These microscale and nanoscale surface features, including holes, pits, and grooves, may affect protein absorption and cell adhesion dynamics, which improve the osseointegration through regulating cell signaling pathways.18–20 Numerous implant surface treatments have already been clinically applied, but detailed scientific evidence provided by manufacturers is limited.21

Strontium (Sr) ranelate acts as an effective drug in clinical treatment of postmenopausal osteoporosis. Sr shows a positive effect on osteoblast differentiation but a negative effect on osteoclast formation.22,23 Sr is a natural bone trace element in the human body and is in the same element group as Ca. Numerous studies have demonstrated that chemical modification of Sr on the implant surface can enhance the osseointegration of the implant.24–30 Recently, it has been demonstrated that a Sr-incorporated Ti surface can promote the recruitment of osteoblasts and osteogenic differentiation of bone marrow–derived mesenchymal stem cells (BMSCs) through the SDF-1α/CXCR4 signaling pathway.31,32 Besides, due to the multiple functions produced by Sr, antiadipogenesis capability and rapid osseointegration are enhanced.33 Previous studies have verified that Sr-incorporated Ti implants promote early bone osseointegration in both osteoporotic and nonosteoporotic rabbits.25,34 It is suggested that Sr-functionalized implants may have potential in promoting new bone formation and thus shorten the period of clinical treatment.

In reality, for the sake of scientific research, SLA-Sr implants should be compared with other commercially available implants before clinical application in order to explore the advantages of Sr. In this study, Sr-incorporated micro-/nano-rough surfaces of Ti implants were made through hydrothermal treatment.32 Surface topographies, quantitative surface roughness, and surface chemical compositions of implants were examined. Meanwhile, the osseointegration of a Sr-incorporated implant and four other commercial implants was assessed using a rabbit model. Additionally, biologic effects were evaluated by removal torque testing, histologic, and histomorphometric analysis after 3, 6, and 12 weeks of implantation, respectively.

**MATERIALS AND METHODS**

**Sample Preparation**

The implants used in this study were four kinds of screw-type commercial implants provided by corresponding manufacturers as shown in Table 1 and one self-developed SLA-Sr implant. The shapes of these five implants are shown in Fig 1. The implants were divided into five groups: RBM (MOZO-GRAU), SLA-1 (OSSTEM), STA (XIVE), SLA-2 (ZDI), and SLA-Sr. The SLA-Sr implants were processed in a laboratory, through hydrothermal treatment in Sr(OH)2·8H2O (99.5% purity; Sigma Aldrich) solution, then cleaned ultrasonically in ddH2O, and dried by N2.32

**Surface Characterization**

The surface topographies of different implants were observed at $1,000, 5,000, 20,000, \times 100,000$ magnifications using a scanning electron microscope (SEM) (SU8010, Hitachi) with an accelerating voltage of 20.0 kV. The chemical composition of five kinds of implant surfaces was examined using x-ray energy-dispersive spectrometry (EDS) (SU8010, Hitachi). Additionally, the SLA-2 and SLA-Sr plates were examined using x-ray photoelectron spectroscopy (XPS) (Escalab250Xi; Thermo Fisher Scientific). During the XPS detection process, Ti plates were shot by the monochromatic Al Kα x-ray (150 W; 15 kV, 10 mA) at a takeoff angle

**Table 1 Information on Implants Included in the Study**

<table>
<thead>
<tr>
<th>Implant</th>
<th>Type</th>
<th>Surface treatment</th>
<th>Diameter/length (mm)</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOZO-GRAU</td>
<td>STD INHEX</td>
<td>Resorbable blasting media (RBM, blasted with CaP ceramics)</td>
<td>4.25/8</td>
<td>Mozo Grau S.A., Valladolid, Spain</td>
</tr>
<tr>
<td>OSSTEM</td>
<td>TS III</td>
<td>Sandblasting with alumina and acid etching</td>
<td>4.2/8.5</td>
<td>OSSTEM IMPLANT, Korea</td>
</tr>
<tr>
<td>XIVE</td>
<td>S plus</td>
<td>Grit blasting and thermal acid etching (neutralization)</td>
<td>3.8/8</td>
<td>DENTSPLY Implants Manufacturing</td>
</tr>
<tr>
<td>ZDI</td>
<td>ZFT-SP</td>
<td>Sandblasting with large-grit and double-acid-etching</td>
<td>4.0/8</td>
<td>Zhejiang Guangci Medical Appliance</td>
</tr>
</tbody>
</table>
of 90 degrees in an ultravacuum environment. The binding energy was rectified with C1s (hydrocarbons C-C, C-H) contribution at 284.8 eV. The chemical crystallographic structures on the SLA-2 and SLA-Sr plates were examined using thin-film x-ray diffraction (XRD) (XRD-7000, Shimadzu) at 1.6 kW power with a Cu-Kα.

The quantitative surface roughness of the tip of implants was detected by three-dimensional (3D) optical microscope (Wyko NT9100, Veeco) at ×50 magnification. The roughness was evaluated by the arithmetic average of the absolute values of the irregularity (Ra), the root mean square of the roughness of the profile (Rq), and the maximum peak-to-valley height of the entire measurement trace (Rt). Six samples in each group were detected.

Animal Model and Surgical Procedure

The animal experiment was conducted with the approval of the Institutional Animal Care and Use Committee of Zhejiang University (ZJU20181169). Six-month-old female New Zealand rabbits weighing 3.1 to 3.3 kg were housed at room temperature with 12-hour light: 12-hour dark circulation and standard rabbit diet. The diet and drink were suspended 4 hours before operation. Forty-five rabbits were randomly assigned into three groups with 15 rabbits in each group: 3 weeks, 6 weeks, and 12 weeks. Then, the 30 hind legs of 15 rabbits were randomly and evenly assigned into 5 small groups (RBM, SLA-1, STA, SLA-Sr, SLA-2). Each hind leg corresponded to two implants from the same group, one in the tibia and one in the femur.

Each rabbit was anesthetized by auricular intravenous injection of 3% pentobarbital sodium (30 mg/kg; West Asia Chemical). After successful anesthesia, the local skin was disinfected with 5% povidone iodine solution and then was draped with the sterile fenestrated sheet. The field of operation was injected with 2% lidocaine hydrochloride to achieve local infiltration anesthesia. The procedure was recorded as follows. A linear incision was made at the proximal tibiae and the femoral condyle, respectively. Then, subcutaneous tissue and the periosteum were bluntly dissected to expose the bone surface. Next, a hole was drilled using the pioneer drill and a sequence of reaming drills with continuous physiologic saline cooling. The preparation of the implant site was conducted according to the protocol provided by the manufacturers. The drilling sequence of RBM was pilot drill D2.0, D3.0, D3.3, D3.8, and profile drill D4.1 at 800 rpm successively. The drilling sequence of SLA-1 was pilot drill D2.2, taper drill D3.5, and D4.0 at 800 rpm successively. The drilling sequence of STA was drill D2.0, D3.0, D3.4, and D3.8 at 800 rpm successively. The drilling sequence of SLA-2 was drill D2.2 (at 800 rpm), D2.7 (at 600 rpm), D3.4 (at 400 rpm), and tapping drill D4.0 (at 15 rpm) successively. Afterward, implants were inserted into the proximal tibiae and the femoral condyle at 15 rpm. Implants of the same group were placed into the same side of the tibia and femur. Eventually, the closed suture was performed in layers. Each rabbit was administered antibiotics intramuscularly (penicillin, 400,000 U/d) for 3 days after operation. Rabbits were sacrificed by overdose anesthetic to acquire specimens after 3 weeks, 6 weeks, and 12 weeks of surgery, respectively.

Removal Torque Testing

The removal torque value (RTV) of the specimens with implants in the femoral condyle was tested immediately using a torsion-testing machine (CTT2500, MTS). The femoral condyle was fixed with 47ºC melting point indium-tin alloy during the test. The measurement was conducted at 5 degrees/min compression speed, and all the peak values of removal torque were recorded by computer.25,34
The samples of proximal tibiae with the implants were stored in 4% paraformaldehyde (Solarbio Technology) for 7 days. Then, samples underwent dehydration and polymerization and were cut into 200-μm slices by cutting machine (300CP; EXAKT). The slices were ground to 20 to 25 μm successively with 320, 800, and 1,200 meshes of sandpaper using a grinding machine (400S, EXAKT). The final thickness of samples was checked by the micrometer. After staining by the methylene blue-fuchsine method, sections were prepared for histologic and histomorphometric analysis. These samples were imaged using a bright-field microscope (DM4000, Lecia) and a PC-based image analysis system (Image Pro Plus). Regions of interest were determined as the surfaces of threads of the implants. The bone-to-implant contact ratio (BIC%) was calculated as the linear percentage of the bone in direct contact with the implant to the entire surface of the implant. The bone area ratio (BA%) was obtained by dividing the sum of bone inside the threads by the entire area between threads. BIC% and BA% in the cortical bone and the cancellous bone were calculated separately.

Statistical Analysis
All data were presented as mean ± standard deviation. Statistical analysis of RTV, surface roughness, BIC%, and BA% between groups was performed using SPSS software (version 20.0, SPSS). The Kruskal-Wallis test was adopted to analyze the data of five groups at the same time point. The Bonferroni correction was used as a post hoc test. \( P < .05 \) was considered as a statistically significant result.

RESULTS

Surface Characterization
At lower magnifications, SEM images (Fig 2) revealed that surface topographies of SLA-1, STA, SLA-Sr, and SLA-2 exhibited numerous irregular microscale pits, which were shaped by SLA with the exception of RBM. RBM exhibited a different lamellar or schistose pattern caused mainly by sandblasting. At the \( \times100,000 \) magnification, massive granular aggregations, and protrusions with an average size of 50 nm were observed on the nanoscale surface of SLA-Sr, compared with SLA-2. In addition, nanoparticles of approximately 15 nm in diameter were scattered on the surface of STA evenly. No nanoparticles were observed on the surface of RBM, SLA-1, and SLA-2.

From EDS analysis (Fig 3a), Ca was detected on the RBM surface. Also, it was verified that Sr was on the SLA-Sr surface rather than the SLA-2 surface. Apart from Ti
and carbon, no other special elements were detected on surfaces of SLA-1, STA, and SLA-2. Moreover, well-defined Sr three-dimensional (3D) core-level spectra were observed on the SLA-Sr plate (Fig 3b) and none on the SLA-2 plates (Fig 3c). In addition, the XRD spectra (Fig 3d) indicated characteristic peaks of Ti on the SLA-Sr plate translated to the left slightly compared with the SLA-2 plate. The peaks of SrTiO$_3$ crystalline (JCPDS card no. 035-0734) were observed on the SLA-Sr plate.

Values of surface roughness parameters varied among different implants as presented in Table 2. By comparing Ra, Rq, and Rt, SLA-1 and STA exhibited significantly greater surface roughness than RBM, SLA-Sr, and SLA-2.
Removal Torque Testing
All rabbits survived. Three weeks after implantation, the RTVs of SLA-Sr (44.98 ± 12.10 Ncm) were significantly higher compared with RBM (24.42 ± 8.14 Ncm, *P* < .05) and SLA-2 (26.81 ± 5.01 Ncm, *P* < .05), as shown in Fig 4. At 6 weeks, the RTVs of SLA-1 (55.97 ± 20.67 Ncm) were significantly higher compared with RBM (27.85 ± 8.50 Ncm, *P* < .05). Furthermore, no significant difference was observed among the five groups at 12 weeks.

Histologic and Histomorphometric Analysis
The overall bone responses were similar among five different surfaces. Newly formed woven bone exhibited crimson color and was in direct contact with the implant surface, filling the gaps between threads 3 weeks after implantation (Fig 5a). The periosteal reaction was evident at this stage in the cortical bone region, and the bone matrix exhibited an irregular interlaced arrangement. In cancellous bone, more continuous trabecula and matrix were noted around implants, especially around SLA-Sr. Then, at 6 weeks, the healing proceeded. The woven bone remodeled into lamellar bone, and the newly mineralized lamellar bone gradually became mature and compact. With bone formation and absorption proceeding simultaneously, the bone remodeling continued slowly. Fully compacted bone reached the implant surface, and a broad bone contact was observed in cortical bone 12 weeks after implantation. In cancellous bone, the majority of primary trabecula disappeared, and only a few of them remained in direct contact with the surface of threads.

As illustrated in Figs 5b to 5m, RBM obtained lower BIC% in cortical bone compared with STA at both 3 weeks (44.48 ± 10.74 vs 74.28 ± 11.50, *P* < .05) and 6 weeks (43.20 ± 11.00 vs 75.11 ± 2.76, *P* < .01) after insertion. At 12 weeks, SLA-1 showed significantly higher BIC% in cortical bone compared with RBM (83.91 ± 4.25 vs 59.93 ± 9.06, *P* < .05). No significant differences were observed among other groups.

Regarding BIC% in cancellous bone, no significant differences were noted among groups during the entire observation period.

SLA-Sr exhibited significantly increased BA% in cortical bone compared with RBM (65.92 ± 15.19 vs 45.93 ± 9.91, *P* < .05) at 3 weeks, whereas no significant difference was observed regarding BA% in cortical bone among all groups at 6 and 12 weeks.

Considering BA% in cancellous bone, no remarkable difference was observed among the surfaces of all groups during the entire experimental period.

DISCUSSION
The bone healing duration varies among different species. For rabbits, the expected time of remodeling is 6 to 7 weeks. In this study, 3 weeks after implantation could be regarded as the early healing period. After 12 weeks post-implantation, the bone remodeling was almost completed.

Macrogometry and Diameter
STA was the narrowest implant in diameter (3.8 mm) among these implants. Hsu et al found that implant diameter did not influence 3D BIC% significantly. Some animal experiments also found no statistical difference
of BIC% or BA% between two sets of implants with different macrogeometries in the tibia.\textsuperscript{39,46} Hence, the effects of diameter of STA on histomorphometric analysis could be neglected to some extent. Nevertheless, studies showed a statistically significant increase of implant stability with increasing implant diameter.\textsuperscript{41,42} Truly, in this study, the RTVs of STA were slightly lower than SLA-1 and SLA-Sr but without significant difference.

Additionally, different implants differ in macrogeometries. RBM, SLA-1, and STA were tapered shapes, but SLA-Sr and SLA-2 were parallel-walled implants. An animal study found no significant difference between tapered and cylindrical implants regarding implant stability quotient values and bone-to-implant contact values.\textsuperscript{43} Besides, a systematic review and meta-analysis showed that tapered implants had higher implant stability values than parallel-walled implants at insertion and 8 weeks but without significant difference.\textsuperscript{44} Therefore, the influence of implant shapes could be neglected, and chemical compositions and surface topographies should be taken into account.

Furthermore, as shown in Fig 1, the neck of RBM implants has unique microthreads. Chowdhary et al observed maximum stress at the first thread in most of the osseointegrated implants.\textsuperscript{35} The effect of microthread application is to enhance bone formation and implant stability.\textsuperscript{45,46} However, RBM obtained lower RTVs and BIC% in cortical bone compared with other groups. Therefore, the influence of other surface characteristics including surface topographies and roughness might have covered up the advantage of the microthreads of RBM implants.

**Chemical Modification**

**Strontium Functionalization.** Compared with SLA-2 and RBM, SLA-Sr performed better in the evaluation of RTV and BA% in cortical bone at 3 weeks, namely, the early stage of bone formation and osseointegration. From 3 to 12 weeks after implantation, the deviations of RTVs, BIC%, and BA% between groups decreased, which indicated the importance of surface treatment in early healing. Consequently, it can be concluded that SLA-Sr possesses a better osseointegration property than SLA-2 during the early healing period.

The advantage has also been demonstrated and interpreted by relevant experiments. In vivo, the Ti-Sr-O coating formed by hydrothermal treatment or a magnetron sputtering process continued releasing strontium steadily. It accelerated bone formation and reconstruction in the early bone healing, which was even better than SLA implants.\textsuperscript{25,27,30,48} Additionally, a study suggested that the Ti-Sr-O coating showed obvious angiogenic and osteogenic effects on BMSCs. MAPK/Erk and PI3K/Akt signaling pathways were involved in facilitating the recruitment of BMSCs and endothelial cells.\textsuperscript{24} Subsequently, the production of alkaline phosphatase and osteocalcin was increased, and osteogenesis-related genes were upregulated in the early stages.\textsuperscript{31,32,49,50}

**RBM.** Relatively speaking, the RBM implant was the widest implant in diameter (4.25 mm) among five groups, but it exhibited low RTV, BIC%, and BA% 3 weeks after implantation. The results were inconsistent with most research as mentioned previously.\textsuperscript{14–17} RBM implants were widely used in clinics, and high survival rates were reported.\textsuperscript{51,52} A possible explanation would be different surface topography and reduced roughness (Ra = 1.19 ± 0.49 µm) compared with other implants. The topographies of the RBM group exhibited in micrographs (at ×1,000 magnification) were similar to the data reported in the literature related to the RBM treatment.\textsuperscript{53,54} However, the results of this study and some others indicated that the surface formed by RBM was not as rough as that by SLA.\textsuperscript{14,53} Similarly, it was demonstrated in an in vivo study that alumina-blasted/acid-etched (AB/AE) implants exhibited better BIC% compared with RBM treatments, including tricalcium phosphate blasting (TCP), TCP and acid etching, AB/AE, and TCP.\textsuperscript{54} Although Ca was impregnated on the surface after sandblasting, the intensity of RBM and weak acid etching might be insufficient to form 3D pores on the surface. In the present study, the results of histomorphometric analysis indicated that RBM treatment was not as satisfying as SLA or strontium modification in accelerating bone formation in early bone healing. Therefore, compared with SLA and strontium modification, RBM was possibly not favorable for immediate loading as suggested in a clinical study.\textsuperscript{41}

**Nanostructures**

Nanogranules on the surface of SLA-Sr created by hydrothermal treatment were composed of crystallized TiO\textsubscript{2} and SrTiO\textsubscript{3} phases with a diameter of approximately 50 nm, which might contribute to the osteoconduction.\textsuperscript{31,32} Intriguingly, with a size of approximately 15 nm in diameter, the nanoparticles on the STA surface were quite different from the previously described pattern on SLA-Sr. Nanoparticles might change the conformation of proteins to expose specific motifs and provide more binding sites for cell adhesion and activation.\textsuperscript{19,55} Nevertheless, detailed correlations between nanostructures and the type of adhered proteins remain unclear. Further studies on characteristics of nanopatterned surface and cell functions are needed.\textsuperscript{56}

**Surface Roughness**

Without nanoscale structures, SLA-1 achieved better RTVs at 3 weeks and better BIC% in cortical bone at 12 weeks compared with RBM. In view of the micrographs
Fig 5a Histologic sections of RBM, SLA-1, STA, SLA-Sr, and SLA-2 implants in rabbit tibiae after different periods at high magnification (×100). The newly mineralized bone was crimson color, and the mature bone was brick red. Scale bars = 200 μm.
and the greatest roughness (Ra = 3.41 ± 0.81 μm), the perfect technique of SLA processed the surface with deep pits on a microscale so that osseointegration was favored.5 Aparicio et al reported that roughness values of Ra ≈ 4.5 μm were beneficial to osseointegration of dental implants in short- and mid-term healing periods.12 However, these data were inconsistent with the wide agreement in literature that implants with an average surface roughness of Ra ≈ 1.5 μm were optimal for better osseointegration and increased screw-out torque.4,11,21 Biomechanical bonding between the implant and bone took a long time; it took weeks for new bone to grow into the irregular surface of the implant. Therefore, before bone interlocking, the implant relied mainly on its macrodesign and the residue bone fragments between implant threads for stability.4 From this study, it could be deduced that compared with RBM, the implant surfaces with high roughness such as SLA-1

Figs 5b to 5m Scatter plots and medians of five groups at 3, 6, and 12 weeks of (b to d) BIC% in cortical bone; (e to g) BIC% in cancellous bone; (h to j) BA% in cortical bone; (k to m) BA% in cancellous bone (n = 6; *P < .05, **P < .01).
and STA might increase the stability due to manufacturers’ different processing parameters. As time went by, the effect of chemical modification on the implant surface decreased and the surface roughness and macrogeometry of implants began to develop the influence gradually. Nevertheless, with the increasing surface roughness, some long-term clinical trials indicated that it might raise the risk of bone loss or peri-implantitis. More reliable studies and evidence are still of high value to identify an optimal surface roughness.

However, some drawbacks existed in the research. The diameters and macrogeometries of different commercial implants were not unified in the study. It was possible that these potential confounders influenced the results of the experiment. Then, surface properties, such as charge, wettability, and contamination, affecting the type and amount of absorbed proteins and cells should also be carefully considered. Moreover, bias could be caused by assigning rabbits with different implant groups in this study. Therefore, more animal experiments such as using a dog model or minipig with more rigorous study design are needed.

CONCLUSIONS

Compared with SLA-2 and RBM, the implant with the strontium-oxide layer displayed slight advantages in new bone formation and osseointegration in the early healing stage in vivo. In the later osseointegration stages, the results of SLA-Sr were comparable with other implants. SLA-1 and STA might have an advantage over RBM in osseointegration due to higher surface roughness.

ACKNOWLEDGMENTS

The authors would like to thank Mozo Grau S.A., Osstem Implant, Dentsply Implants Manufacturing, and Zhejiang Guangci Medical Appliance for the donation of commercial implants. This study was financially supported by National Natural Science Foundation of China (No. 31670970), Natural Science Foundation of Zhejiang Province of China (No. Y15H140003), Key Research and Development Program of Science and Technology, Department of Zhejiang Province (No. 2019C03081), and Research foundation of Department of Health of Zhejiang Province (No. 2018KYS00). The authors reported no conflicts of interest in this study.

REFERENCES


