Effect of a Multiphasic Anodic Spark Deposition Coating on the Improvement of Implant Osseointegration in the Osteopenic Trabecular Bone of Sheep

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***Purpose**: Anodic spark deposition techniques have been effectively applied to achieve a microporous morphology on metals. To investigate the effect of a new anodic spark deposition–based treatment in the enhancement of titanium implant osseointegration in trabecular bone of aged and ovariectomized sheep, a histomorphometric and microhardness study was carried out. **Materials and Methods**: Ten sheep were divided into 2 groups. Five were submitted to a bilateral ovariectomy to induce an estrogen-deficiency osteopenia (Ovariectomized), and 5 were left untreated (Aged). Twenty-four months later, they underwent a bilateral implantation of commercially pure titanium screw threads in the lateral surface of femoral condyles: electrochemically treated titanium (SP) and acid-etching treated titanium (BioRough). Twelve weeks after the second operation, the animals were sacrificed and femur segments and iliac crest biopsy specimens were examined for histomorphometric and microhardness evaluations. **Results**: The histomorphometry of the trabecular bone of the iliac crest biopsy specimens and that around screws showed marked signs of bone rarefaction in the Ovariectomized group when compared to the Baseline and Aged groups. Significantly greater bone-implant contact was observed for SP implants in comparison with BioRough implants in both the Aged ($P < .001$) and Ovariectomized ($P < .01$) groups. No significant differences in terms of microhardness were found between SP and BioRough implants within the Aged group, while a significantly higher Bone Maturation Index was observed for SP in the Ovariectomized group ($P < .05$). **Conclusions**: The novel electrochemical treatment SP produced the most promising results and was able to introduce substantial improvements in achieving the fast and stable osseointegration of implants in osteopenic bone.* Int J Oral Maxillofac Implants 2008;23:659–668

Key words: *anodic spark deposition, electrochemical, osteopenia, titanium*

Different systemic conditions are known to be responsible for causing a progressive structural deterioration of the bone tissue, thus leading to bone fragility, either directly or due to the therapies used for their treatment. Senile and postmenopausal osteoporosis are among the most important conditions and have important consequences on the success of fixation devices, prostheses for total joint replacement, and dental implant surgery.[1] Bone alterations due to age and estrogen deficiency are both structural and biological. High-quality bone seems to be important for the initial stability of implant devices, and changes in structural and biomechanical properties due to bone rarefaction and microarchitectural deterioration are responsible for reduced implant stabilization.[1] The slowing down of the biomaterial osseointegration processes, which is essentially a wound-healing process, has been found to be due to biological drawbacks. In fact, both aging and estrogen deficiency modify cell proliferation, synthetic activity, reactivity to local and systemic factors, mesenchymal stem cell number, and skeletal content of anabolic and catabolic cytokines.[2] Therefore, a reduction in osseointegration rate of many biomaterials when implanted in osteopenic bone, both in cortical and trabecular sites, had been demonstrated both in dental and orthopedic reconstructive surgery.[1–8]

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Various surface modification and deposition techniques have been developed and applied to metals used for dental and orthopedic implants to produce microrough titanium surfaces for fast and durable osseointegration as well as implant stability over time.[9–11] The success obtained with microtopographies has prompted researchers to study in detail the role of nanotopographies in improving osseointegration, and interest in the development of nanostructured surfaces for dental and orthopedic implants is increasing.[12–17]

Among surface modification techniques, electrochemical techniques, such as anodic spark deposition (ASD), have been effectively applied to achieve a microporous morphology on titanium and titanium alloy surfaces while modifying their surface oxide film.[18–22] In the same way, acid-etching techniques, alone or combined with sandblasting, have offered the big advantage of better control over the surface cleanness by decontaminating the implant surface and thus demonstrating superior resistance to reverse torque.[23–26]

Recently, a new electrochemical process has been developed to improve further the mineralization potential, mechanical stability, and corrosion resistance of the ceramic coating obtained with ASD.[27,28] The new process consists mainly of 2 consecutive ASD processes, the first performed in a phosphate solution and the second in a calcium solution, followed by an additional alkali etching step, and finally by a fast mineralization in simulated body fluid (SBF). Sandrini et al concluded that nanostructured titanium oxide (TiO_2) obtained by the new process combines all the prerequisites required of a ceramic coating: mineralization potential, preferential protein adsorption, and osteoblast-activity-stimulating potential.[29] Chiesa et al observed that the new process enhanced titanium bioactivity and osseointegrative properties when treated implants were placed implanted in trabecular bone, without introducing any detrimental effects on the mechanical properties of the material.[30] However, it is still unknown whether aging and estrogen deficiency influence peri-implant bone healing and the clinical success rate implants treated with this new electrochemical process.

The aim of the present study was to investigate in a large-sized animal model after unloaded implantation the effect of the new electrochemical biomimetic treatment in the enhancement of implant osseointegration in the trabecular bone of aged and ovariectomized sheep by comparing histomorphometric and microhardness measurements with an acid-etched titanium surface and commercially pure titanium.

MATERIALS AND METHODS

Sample Preparation

Screw-type implants made of commercially pure grade 2 titanium (CpTi; ISO 5832-2), 12 mm in length and 4 mm in diameter machined and prepared on a turning lathe (CpTi, $R_a = 0.47 \pm 0.01$ μm) were used as controls and substrates for other surface treatments. All implants were cleaned by ultrasonic rinsing (Branson Automatic Cleaner, [Branson Corp, Danbury, CT]) in acetone (RPE Carlo Erba, Milan, Italy) for 5 minutes, then in distilled water for an additional 5 minutes, to degrease and remove contaminants from the surface.

The commercially pure titanium implants were submitted to an electrochemical surface treatment performed by 2 consecutive ASD processes carried out in different electrolyte solutions at different voltage ranges, and followed by an alkali etching process (SP), while other CpTi implants underwent an acid-etching process (BioRough).[28–30] Briefly, SP implants were placed in an electrochemical cell at 0°C (± 2°C), with 2 different electrolyte solutions (ASD1, phosphate anions and calcium cations; ASD2, only calcium cations) at 2 different voltage ranges (ASD1, 0 to 350 V at 70 A/m^2; ASD2, 0 to 370 V at 35 A/m^2). They also underwent an alkali etching process in concentrated KOH (60375, Fluka, Chemika, Italy) water solution at 60°C ($R_a = 0.29 \pm 0.03$ μm). BioRough implants were prepared by a double-step etching process (NanoSurfaces; proprietary process). An initial alkali etching performed at 80°C was followed by a second etching step consisting of an acid treatment performed at 28 ± 2°C for 1 hour ($R_a = 1.07 \pm 0.09$ μm).

Physicochemical and Morphological Properties Analysis

The roughness parameters were calculated on 1.5-mm-long profiles. Measurements were acquired using a 3D laser profilometer (UBM-Microfocus Compact, Nanofocus, Ettlingen, Germany), and every measurement was repeated 5 times.

The morphology of the different surfaces was analyzed by means of a scanning electron microscope (SEM, STEREOSCAN 430; Leica Cambridge Mirosystems, Milton Keynes, UK) equipped with a backscattered electron detector. All samples investigated with SEM were sputter-coated with gold (Sputter Coater SC7640, Polaron). Non–sputter-coated surfaces were also investigated by electron dispersion spectroscopy EDS, Inca Energy 200 analyser, with Si[Li] detector PENTAFET 4, Great Britain) and the software package ZAF-4/FLS for massive sample analysis.

Surface properties were investigated with thin film x-ray diffraction (TF-XRD, Siemens D500 Kristalloflex)

at 40 mA and 40 kV to achieve better knowledge of the crystalline structure of the differently-treated surfaces and to assess the presence of crystalline titanium oxides (TiO_2) and other oxides, as a result of oxidation processes on the titanium surface. Different XRD patterns of the specimens were already presented and discussed in a previous study.[30]

Study Design

This study was performed according to European and Italian law on animal experimentation and the principles stated in the "Guide for the Care and Use of Laboratory Animals."[31]

Ten crossbred (Bergamasca-Massese) sheep 70 ± 5 kg body weight were used. The animals were fed a standard diet (Mucedola, Settimo Milanese, Italy) containing 0.85% calcium and 0.51% phosphorus and allowed tap water ad libitum. All surgical procedures were performed under general anesthesia following a standardized protocol as well as postoperative therapy.[8] After the recovery period, the animals were returned to external breeding in the same free natural conditions and were fed the same standard diet described above.

Ten animals were divided into 2 groups: 5 animals aged 9 years at the moment of implantation surgery that had been ovariectomized 24 months before to induce estrogen deficiency (Ovx group) and 5 animals aged 9 years at the moment of the implantation surgery (Aged group). All the animals underwent a bilateral implantation of prepared screw threads in the lateral surface of femoral condyles. Three holes 3.9 mm in diameter, transversally oriented, were drilled at low speed under sterile 0.9% NaCl. Then the holes were flushed and cooled with sterile 0.9% NaCl to remove bone debris and 1 implant was placed in each hole. One screw thread for each surface treatment was tightened in the femoral condyle. A transiliac biopsy of the iliac crest was performed vertically during both surgery procedures (ovariectomy and implant surgery) in all of the animals[32] to evaluate the bone status and thus to study the performance of the tested surface treatment according to the quality of host bone.

Twelve weeks after the second surgery, the animals were pharmacologically euthanized under general anesthesia, and the femurs were excised and stripped of soft tissue. Cubic bone segments from the femoral condyles, each containing an implant, were obtained using the EXAKT B System 300 CL for cutting (EXAKT Apparatus, Norderstedt, Germany). The bone segments and the iliac crest biopsy specimens were fixed in 4% paraformaldehyde for 24 hours for histologic and histomorphometric evaluations carried out by blinded operators.

Histologic Evaluation

The bone segments and iliac crest biopsy specimens were dehydrated in a graded series of ethanols and embedded in polymethyl methacrylate. Undecalcified 60-µm-thick bone-implant sections obtained parallel to the long axis of the implant and 20-µm-thick iliac crest biopsy sections were obtained using a Leica SP 1600 diamond saw microtome cutting system (Leica, Milan, Italy). Sections were stained with fast green, acid fuchsin, and toluidine blue. Histomorphometric analyses were performed using an optic microscope (BX41, Olympus Optical, Europa, Germany) connected to an image analyzer system (Qwin; Leica Imaging Systems, United Kingdom).

The following histomorphometric parameters were measured on both sides of the implants:

- Percentage of bone-implant contact (%BIC): the amount of bone contact at the interface, defined as the percentage of implant length with direct BIC without intervening soft tissue layers[33]
- Bone ingrowth (%): the amount of newly formed bone tissue (woven and lamellar bone) expressed as a percentage measured inside the threads in an area located between the bottom and the top of the thread[34]

The following static histomorphometric measurements were performed and calculated in the iliac crest and femoral condyle trabecular bone around implants[35]:

- Trabecular bone volume (BV/TV, %): the whole spongy bone area present expressed as a percentage of the total tissue area (T.Ar)
- Trabecular thickness (Tb/Th, µm): given by 1.199 * spongy bone area/2/B.Pm, where 1.199 is used for correcting section obliquity
- Trabecular number (mm): BV/TV * 10/trabecular thickness
- Trabecular separation (µm): 1,000/trabecular number–trabecular thickness

Microhardness

The resin-embedded blocks containing the implanted screws were used to measure bone hardness by an indentation test (Microhardness VMHT 30; Leica, Wien, Austria).[8,36] The microhardness (HV) measurements were taken tangentially to the interface with a Vickers indenter applied to the bone at a load of 0.05 kgf and a dwell time of 5 seconds. The average value for each sample was calculated on a mean of 10 measurements for each examined area at 2 sites: *(a)* in the regrown bone within 200 µm from the interface and in the inner area in which the threads

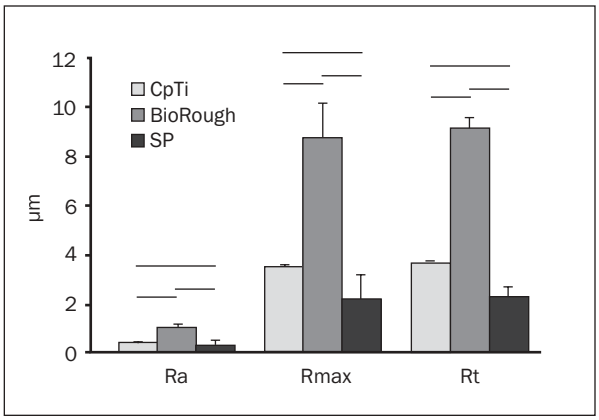
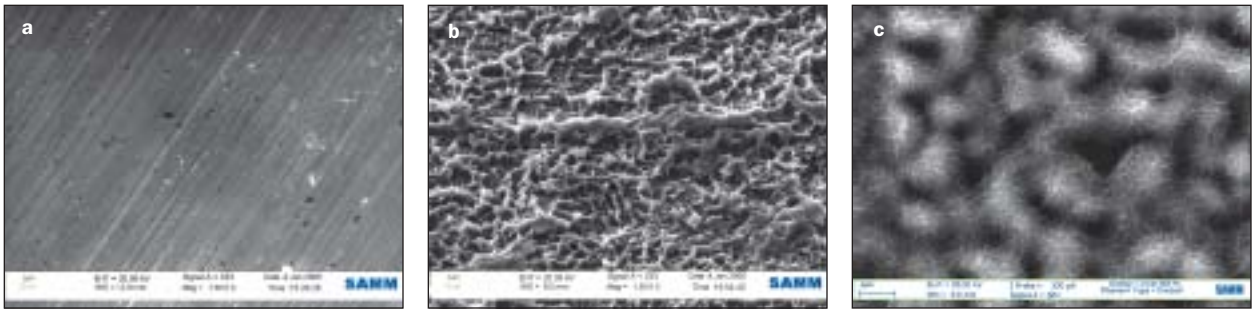


Fig 1 Surface roughness for the 3 different surface treatments (mean ± SD, n = 5). Scheffé multiple comparison test ($P < .001$) for all comparisons.

Fig 2 High-magnification secondary electron emission micrographs of *(a)* the CpTi implant surface; *(b)* the BioRough implant surface and *(c)* the SP implant surface.



of the screw engage ($HV_{200µm}$) and *(b)* outside the threads in the pre-existing host bone at 1,000 µm from a line connecting the top of the threads ($HV_{1,000µm}$). Finally, the bone maturation index (BMI) was calculated by dividing the microhardness of the bone re-grown at the interface by the microhardness of the pre-existing bone multiplied by 100.

Statistical Analysis

Statistical analysis was performed using the SPSS v.12.1 software (SPSS, Chicago, IL). After the normal distribution and the homogeneity of the variance were verified, the 1-way ANOVA test, followed by Scheffé multiple comparison test, was used to assess significant differences in the histomorphometric parameters of the iliac crest. The Student *t* test was used to compare histomorphometric and microhardness data on implant osseointegration in trabecular bone from femoral condyles. Data were reported at a significance level of $P < .05$.

RESULTS

The SP surface exhibited the lowest average roughness (Ra) whereas BioRough showed the highest values (Fig 1). The Rmax and Rt parameters (RT = the distance between the highest peak and the lowest valley of the whole sample) followed the trend of Ra, with the BioRough surface exhibiting the highest values. It should be recognized that the laser profilometer used to perfom roughness measurement possesses a lateral resolution up to 1 micrometer; it could not pick up nanometric roughness morphology or submicrometric porosity of the SP treatments.

The SEM images of implant surfaces did not exhibit any macroscopic defects; implants appeared well machined, and neither chips nor overlapping of ridges resulting from the machining process were observed (Fig 2). CpTi presented a regular texture due to the machining process (Fig 2a). The BioRough texture was characterized by high and sharp edges with deep valleys (Fig 2b), while SP texture was more regular than titanium and many round crests and deep pores were clearly visible on the surface (Fig 2c). Its whole surface was covered in a thin nanostructured texture overlapping the microporous morphology formed by the ASD process, which was formed only after alkali treatment.[28,29]

EDS analysis indicated that the cleaning procedure was effective in preventing machining oil contaminants from spoiling the surfaces. The smooth CpTi and BioRough implants exposed an external layer of pure titanium, whereas the anodically treated SP surface exhibited high oxygen content enriched by Ca and P (Table 1).

Table 1 Elemental Atomic Composition Recorded by EDS

	O%	Al%	P%	Ca%	Ti%
CpTi	4.85	0.10	(−0.12)*	(−0.01)*	95.18
	6.74	0.11	0.04	(−0.01)*	93.12
BioRough	5.35	0.09	0.06	0.05	94.58
	4.75	0.10	(−0.05)	(−0.03)*	95.46
SP	67.02	0.12	0.32	0.37	32.16
	67.23	0.30	0.34	0.35	31.79

*The negative values around zero and inside the analysis precision range are generated by the EDS analysis software and should be considered zero.
For each material one sample was analyzed on two different areas. Voltage = 20 kV; current = 500 pA; calibration sample = Co; area of analysis = 1 mm^2. Analysis precision within ± 0.15%.

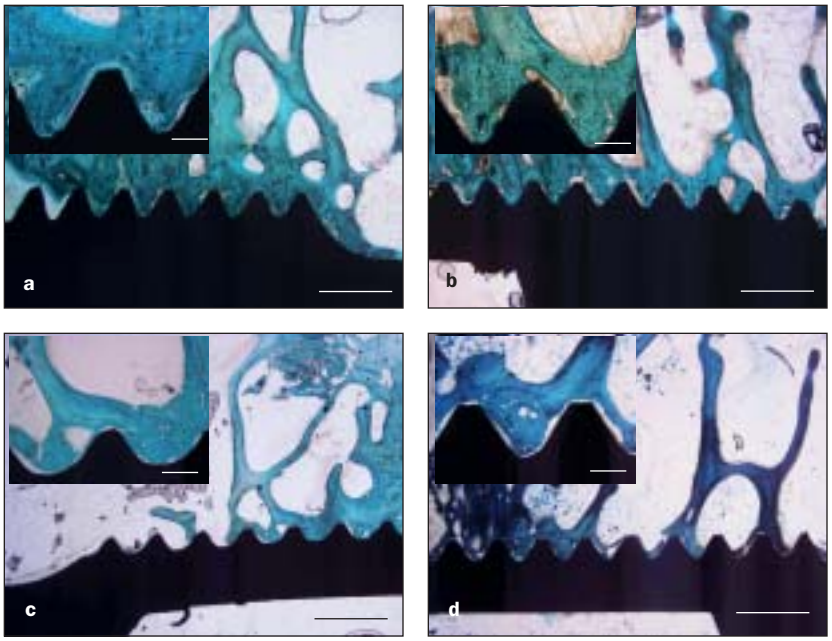


Fig 3 Histologies of new bone in-growth on implant surface after 12 weeks of in vivo implantation of SP *(a, b)* and BioRough *(c, d)* implants in the Aged *(a, c)* and Ovx *(b, d)* groups. Remodeling bone tissue showed good BIC at the resolution level of the light microscopy (×10, scale bar = 100 μm).

No crystalline oxides or other crystalline structures, apart from those typical of titanium, were detected by thin film-XRD analysis of smooth CpTi and the BioRough surfaces. In particular, SP surface was covered in a thick layer of crystalline anatase TiO_2 with traces of rutile. TF-XRD analysis carried out on ASD1 and ASD2 samples showed that the second anodization step of SP treatment was the most effective in achieving crystalline TiO_2 growth.[28–30] The comparison of ASD2 spectra and TiSpark final spectra showed that alkali treatment slightly modified surface crystalline phases, thus favoring the growth of rutile phase.[28–30]

All animals tolerated surgery well and survived the postsurgical period without any local or systemic complications. When dissecting the femoral condyles, neither macroscopic mal-positioning nor signs of infection were observed around any of the implants.

The histologic findings confirmed that all samples were implanted correctly in the trabecular bone of femoral condyles, and neither inflammatory cell infiltrate nor signs of infection were observed.

Both SP and BioRough implants showed a newly formed peri-implant bone regularly arranged in a 3-dimensional network that filled the gap between the pre-existing trabecular bone and the implants (Fig 3). The amount of the bone trabeculae seemed to be more developed for CpTi samples, with more numerous bone trabeculae in direct contact with the implant surface. In the Ovx group, decreases in the bone volume, bone apposition, and trabecular bone thickness were observed around all implants (Fig 3b and 3d).

The histomorphometric analysis of the iliac crest biopsy specimens showed that the trabecular bone volume (BV/TV) of the Ovx group decreased significantly when compared to the baseline (–42%) and

Table 2 Histomorphometric Parameters of Iliac Crest Bone in Aged and Ovx Animal Groups (Mean ± SD, n = 5)

Parameter	Unit	Baseline (n = 10)	Aged	Ovx
BV/TV	%	38.05 ± 2.7	33.28 ± 2.9**	22.2 ± 3.1***,°°°
Tb.Th	µm	124.5 ± 20.8	108.1 ± 7.0*	68.9 ± 15.3***,°°
Tb.N	/mm	3.1 ± 0.4	3.1 ± 0.3	3.3 ± 0.4*,°
Tb.Sp	µm	202.1 ± 25.9	218.6 ± 31.4**	238.7 ± 19.8***,°°°

BV/TV = trabecular bone volume; Tb.Th = trabecular thickness; Tb.N = trabecular number; Tb.Sp = trabecular separation.
Scheffé multiple comparison test of Aged and Ovx groups versus Baseline: *$P < .05$; **$P < .005$; ***$P < .001$. Ovx versus Aged groups: °$P < .05$; °°$P < .005$; °°°$P < .001$.

Table 3 Histomorphometric Results of Femoral Condyle Trabecular Bone Around Screws for Aged and Ovx Groups (Mean ± SD, n = 5 duplicate)

Parameter	Unit	Aged	Ovx
BV/TV	%	39.7 ± 3.8	22.0 ± 4.0***
Tb.Th	µm	219.8 ± 53.6	178.6 ± 15.8
Tb.N	/mm	1.6 ± 0.4	1.0 ± 0.2
Tb.Sp	µm	441.5 ± 97.0	811.7 ± 148.5*

BV/TV: trabecular bone volume; Tb.Th: trabecular thickness; Tb.N: trabecular number; Tb.Sp: trabecular separation.
Unpaired Student *t* test: *$P < .05$; ***$P < .001$.

Table 4 Histomorphometric and Microhardness Results of CpTI Implants in Aged and Ovx Groups at 12 Weeks (Mean ± SD, n = 5 duplicate)

Parameter	Unit	Aged	Ovx
BIC	%	38.6 ± 5.4	28.8 ± 3.5 *
BI	%	76.0 ± 7.8	59.2 ± 3.8 **
$HV_{200µm}$	Vickers	48.9 ± 1.7	43.6 ± 0.6 **
BMI	%	90.3 ± 3.1	80.4 ± 1.1 **

BIC: bone-implant contact; BI: bone ingrowth; $HV_{200µm}$ = regrown bone hardness; BMI = bone maturation index.
Unpaired Student *t* test: *$P < .05$; **$P < .005$.

the Aged (–33%) groups (Table 2). The trabecular thickness (Tb.Th) decreased by 45% ($P < .001$) and 36% ($P < .005$) in the Ovx group in comparison with the Baseline and Aged groups, respectively. Finally, the trabecular separation (Tb.Sp) of the Ovx group increased significantly by 18% in comparison to the Baseline group. Even the trabecular bone around the screws showed signs of bone rarefaction in the Ovx group. In particular, the BV/TV (–45%) and Tb.Sp (84%) parameters were significantly different from those of the Aged group (Table 3).

The histomorphometric and microhardness results of CpTi implants in the Aged and Ovx groups are reported in Table 4; significant differences were found for all investigated parameters and the lowest values were obtained in the Ovx group. To minimize any possible errors in the evaluation of data, the results obtained from SP and BioRough implants were compared within the same experimental group and between groups, as differences the CpTi surface was considered a control.

Both SP and BioRough implants gave higher values than CpTi in terms of BIC and bone ingrowth (BI; Table 5). Significantly higher ΔBIC results were observed for SP implants in comparison to BioRough in both the Aged ($P < .001$) and Ovx ($P < .01$) groups, while significant differences between experimental groups were found only for ΔBIC of SP implants ($P < .05$). With regards to the ΔBI results, significantly higher values were found for both implants in both experimental groups. In addition, significant differences ($P < .005$) were found between experimental groups for ΔBI of both surface treatments.

Estrogen deficiency and implant osseointegration did not alter the microhardness of the pre-existing trabecular host bone outside the threads ($HV_{1,000µm}$: Aged: 54.8 ± 1.2, n = 15; Ovx: 52.9 ±1.7, n = 15). No significant differences in terms of $\Delta HV_{200µm}$ and ΔBMI were found between SP and BioRough implants within the Aged group, while a significantly higher ΔBMI value for SP was observed in the Ovx group ($P < .05$; Table 6). Significant differences between experimental groups in terms of $\Delta HV_{200µm}$ ($P < .01$) and ΔBMI ($P < .005$) were highlighted only for SP implants (Table 6).

DISCUSSION

The present study aimed at investigating the effect of the new electrochemical-based process, consisting of 2 consecutive ASD processes, followed by an additional alkali etching step, on implant osseointegration by taking into account the quality of the host

Table 5 Bone-Implant Contact (BIC) and Bone Ingrowth (BI) Results for Type of Implants in Aged and Ovx Groups at 12 Weeks Expressed as the Difference with Those Obtained with CpTi (Mean ± SD, n = 5 duplicate)

	ΔBIC (%)			ΔBI (%)		
Surface	Aged	Ovx	Unpaired Student *t* test	Aged	Ovx	Unpaired Student *t* test
SP	31.4 ± 4.2	20.4 ± 6.8	$t = 3.10$, $P < .05$	10.2 ± 5.1	25.5 ± 3.3	$t = -5.63$, $P < .001$
BioRough	14.1 ± 2.3	9.6 ± 10.3	$t = 0.96$, NS	2.4 ± 3.9	14.8 ± 4.2	$t = -4.84$, $P < .005$
Paired Student *t* test	$t = 10.7$, $P < .001$	$t = 5.5$, $P < .01$		$t = 11.0$, $P < .001$	$t = 3.5$, $P < 0.05$	

Table 6 Microhardness Results of Femoral Condyle Trabecular Bone Around Screws for Aged and Ovx Groups at 12 Weeks Expressed as Difference with Those Obtained with CpTi (Mean ± SD, n = 5 duplicate)

Surface	$\mu HV_{200\mu m}$ (Vickers unit)			ΔBMI (%)		
	Aged	Ovx	Unpaired Student *t* test	Aged	Ovx	Unpaired Student *t* test
SP	−1.5 ± 1.5	3.3 ± 2.6	$t = -3.55$, $P < .01$	−3.7 ± 4.2	7.2 ± 1.3	$t = -5.45$, $P < .005$
BioRough	−1.9 ± 0.9	−1.8 ± 2.9	$t = -0.07$, NS	−3.5 ± 1.8	−3.3 ± 5.3	$t = -0.07$, NS
Paired Student *t* test	$t = 0.50$, NS	$t = -0.10$, NS		$t = 2.19$, NS	$t = 4.44$, $P < .05$	

bone. CpTi, SP, and BioRough implants were implanted in aged and estrogen-deficient sheep in order to obtain data on the osseointegration rate of the biomaterials. Generally, it can be said that the surface obtained by the electrochemical process (SP) was better osseointegrated than that obtained by the BioRough process, and that osteopenia, despite reducing the osseointegration capabilities of the implants, does not appear to change the SP surface greatly. These results support the hypothesis of increased osseointegration properties of Ti surfaces due to the development process of a nanostructured TiO_2 obtained by the electrochemical process.

The present findings on the new biomimetic SP surface treatment have added to the previous data on physicochemical and morphological properties, and the in vitro and in vivo behavior of Ti, BioRough, and SP reported in other papers.[29,30,37,38] It was shown that the new electrochemical process supported cell adhesion and viability as well as the CpTi and BioRough surfaces but specifically enhanced cell proliferation. Its unique properties included a bioactive microporous and nanotexture Ca- and P-enriched thick TiO_2 layer with a predominant crystalline anatase structure. As is already known, the catalytic properties of anatase play an important role in the in vitro hydroxyapatite nucleation, which is also related to the high in vivo osseointegration performance,[39–41] and this doping process becomes the basis for the intimate bonding formation between bulk titanium and the bone tissue. Previously, in vivo results obtained in sheep trabecular bone (femoral condyles) indicated that the new electrochemical process positively affected and improved the osseointegration process of titanium implants at only 4 weeks and achieved the greatest osseointegration at 8 weeks after surgery.[30]

More precisely, current histomorphometric results showed that both surface treatments enhanced the implant osseointegration in the Aged and Ovx groups at 12 weeks from surgery, when bone remodeling around implants starts, in comparison with CpTi. Particularly, SP had values around twice as high and significant in comparison with BioRough. Also the results of ΔBIC and ΔBI achieved with the same surfaces implanted in the trabecular bone of healthy sheep were higher in SP (ΔBIC = 32.3 ± 4.3; ΔBI = 9.2 ± 5.3) in comparison with BioRough (ΔBIC = 14.4 ± 2.4; ΔBI = 1.4 ± 3.7).[30] This shows how a substantial difference exists in the osseointegration of the 2 types of surfaces, always greater for SP, how this tends to remain constantly greater in the Aged and Ovx groups, and how the bone growth inside the threads, which is mostly conditioned by the osteo-

penia,[34] is even greater in the Ovx group in comparison with the Aged group ($P < .001$).

Weng et al showed in a canine model that a double acid-etched implant surface can achieve a significantly greater BIC compared to a machined surface when implanted in trabecular bone,[42] and other clinical and experimental studies have shown the positive effect of a single or double acid-etching process on implant osseointegration.[10,11,18,24,25] The current results achieved with the new electrochemical process, doubling those of the double acid-etching one, could be of great interest for patients with biological alterations or a poor bone stock because of a previously failed implant.

Microhardness results were the consequence of the 2 different types of regrown bone around SP and BioRough surfaces (Fig 1): lamellar bone versus woven bone. The microhardness and BMI data indicated that the regrown bone around SP implants was more mature than that formed around BioRough, always in comparison with the control material CpTi, thus suggesting also better stability with this type of implant compared with BioRough, especially in the Ovx group.

Regarding the experimental animal model used, it clearly demonstrated that aging and osteopenia significantly decrease the osseointegration rate of metallic implants.[4,5,7,34] The ovariectomized sheep, which had already been widely used for endocrinologic veterinary studies,[43,44] was proposed as a model of human osteoporosis, because it seemed promising for studying bone turnover, the therapeutic effects of innovative pharmacologic treatments, and biomaterial osseointegration in postmenopausal osteoporosis.[45,46] Recently, other authors have also observed that bone architecture from young and 8- to 13-year-old sheep had considerable age-dependent structural changes due to significant osteopenia and very similar to those found in elderly humans.[47]

An exhaustive review of animal models for fracture treatment in osteoporosis suggested that no animal model of osteoporosis is fully representative of the clinical situation, even though the sheep is still preferred because of its size and the results of studies demonstrating the development of osteopenia after estrogen deficiency.[48,49] The histomorphometric analysis performed on the iliac crest biopsy specimens showed a generalized significant bone rarefaction of trabecular bone in both groups due to aging for the Aged group and due to estrogen deficiency for the Ovx group. This bone rarefaction was significant for BV/TV, trabecular thickness and trabecular separation and, only in the Ovx group, for trabecular number (Table 1). As expected, bone rarefaction was more severe in estrogen-deficient animals than that observed in physiologic aging. Furthermore, the development of osteopenia at the femoral condyle level around screws was worse than that found in the iliac crest during the second operation (Table 2).

The experimental time and implant sites were selected on the basis of previous studies, also taking into account that biological bone response to implants depends on the material properties and surgery trauma.[30] In particular, the forces acting on condylar trabecular sites, which are a combination of shear and compression forces, have been demonstrated to have a beneficial effect on the bone remodeling process of the bone-implant interface.[50]

Microhardness tests provide important information on various bone characteristics, such as calcification degree, arrangement of collagen fibers, mineral quantity per volume unit, elastic modulus and, recently, information on bone-material interface to assess implant osseointegration.[35,51–53] The use of the same embedding blocks for histology and bone microhardness measurements obviated the need for additional specimens for mechanical torsion test. Even though bone fixation and infiltration processes are recognized to increase bone microhardness over time,[51] the use of standardized procedure to fix and infiltrate bone can improve measurements and results. Nevertheless, if an increase in bone hardness occurred, it would have been recorded by both $HV_{200\mu m}$ and $HV_{1,000\mu m}$ measurements and neutralized in the case of BMI-normalized parameters.

In accordance with the increasing age of the population, the number of patients with postmenopausal or senile osteoporosis has increased as well as the number of edentulous patients who may require dental implantation. In fact, osteoporosis has been suggested to contribute to the severity of alveolar bone loss and the eventual loss of multiple teeth.[54] Today dental implants offer the opportunity to edentulous patients to be treated with functional and esthetic alternative reconstructions. The use of endosseous implants in dentistry is increasing thanks to the data on long-term clinical success rates, and this success has prompted the use of implants in several clinical situations, such as deficient bone stock. From an orthopedic point of view, aged and osteoporotic patients often develop fractures that in some cases require the implantation of screws, pins and prostheses. Blomqvist et al demonstrated in a retrospective study (of patients who received sinus-floor bone grafting and implants) that the risk of implant failure is dependent on bone mass density.[55] Becker et al found a significant association between mandibular bone quality and dental implant failure.[56] In a prospective randomized trial,

Barrios et al found that independently of the device used, patients with unstable trochanteric hip fractures and osteoporotic bone had the highest risk of implant failure.[57] The current results achieved with the new electrochemical process, doubling those of double acid-etching one, could be of great interest for patients with structural and biological alterations or a poor bone stock because of a previously failed implant.

CONCLUSIONS

The in vivo tests of this study indicated that both surfaces analyzed could be suitable for endosseous implants. However, the novel electrochemical treatment, SP, exhibited the most promising results and proved to have the potential to introduce substantial improvements for the achievement of fast and stable osseointegration of implants even in osteopenic bone conditions.

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