This study evaluated bone behavior during dynamic osseointegration. A total of 12 implants were placed in sheep tibia and analyzed at 15, 30, 60, and 90 days. Quantitative and qualitative bone behaviors were evaluated with histologic, histomorphometric, Alizarin Red S, and SEM-EDX (scanning electron microscopy with energy-dispersive x-ray spectroscopy) analysis. Twenty microanalyses were performed in chambers 1, 3, and 5 (a chamber is the distinctive space/bone volume between two coils of the implant screw) in distinctive zones: the titanium-bone interface (zone A), the middle chamber–bone front (zone B), the bone–surgical threading interface (zone C), and native bone (zone D; used as a control). The dynamic osseointegration index (DOI) and bone quality index (BQI) with calcium/phosphorus (Ca/P) content were detected to evaluate the osseointegration quality, bone-to-implant contact (BIC), and bone density around implants. At 15 days, initial bone formation with osteoid matrix deposition and different color intensities were observed (means: BIC = 23.3% ± 3.9%; DOI = 1.55). SEM-EDX analysis showed low mineralized bone/bone marrow with a very low Ca/P mean value. At 30 days, high new bone deposition with higher color intensity in the crestal portion was recorded (BIC = 77.3% ± 0.4%; DOI = 2.58). At 90 days, tight BIC to the middle and apical implant portions were detected, as well as several osteon structures in the crestal portion (BIC = 86.4% ± 0.6%; DOI = 0.96). During all observed time periods, the BQI showed 25% more Ca/P in zone A. Greater maturation degree and lower BQI were seen at zone A compared to the other zones. After 15 and 30 days, zones B and C (except for P in zone B) showed BQIs slightly over 50% and around 75%, respectively, confirming a progressively higher degree of bone maturation that proceeds with the osseointegration process. After 90 days, the BQI values of zones B and C (greater than 70% in zone B and around 90% in zone C) confirmed the bone mineralization and maturation process and an acceleration of implant osseointegration, while a lower BQI value (25%) was recorded in zone A. This study shows osseointegration as a variable dynamic process with a higher bone deposition in contact with the implant surface during the early phase, while in the active and later osseointegration times, the bone quality maturation showed higher values only “at distance” (growth of native bone to the implant surface, observed later in the osseointegration process). After 3 months (before loading), the BQI evaluation was lower (25%) in zone A, confirming that the healing and maturation process of the bone cannot be considered complete. Int J Periodontics Restorative Dent 2023;43:65–72. doi: 10.11607/prd.6139
Fifty years after the Brånemark and Schroeder discoveries, implantology is a successful treatment for edentulous rehabilitation, and osseointegration is defined as a direct bone-to-implant interface without fibrous tissue interposition. To obtain predictable clinical success, several studies reported that bone-to-titanium implant interaction was related to chemical properties, surgical technique, and loading conditions. In the last 30 years, osseointegration was considered a clinical interface between hard tissues and the implant without mobility and pain. Several authors suggested guideline criteria of osseointegration success, survival, and failure to determine the implant quality of health, based only on clinical evaluation, 2D or 3D radiographs, or a clinical mobility test. However, Albrektsson and Jacobsson reported that osseointegration can only be evaluated histologically with light microscopic analysis. In the last 20 years, several surface modifications were promoted to reduce healing time and increase the bone-to-implant interface. According to Buser et al and Schenk and Buser, about 60% to 70% of the implant surface is covered by bone, and thus the term “bone-to-implant contact” (BIC) became widely used to measure the degree of osseointegration. In a 2002 animal study, Trisi et al suggested that bone quality plays a crucial role in the BIC percentage. In 2005, Schwartz et al determined that primary stability during 3 to 4 weeks of osseointegration was a critical factor to indicate success. Coelho et al determined the same in 2015.

Even today, there is no agreement on the role of an implant surface in osseointegration, the bone behavior during different phases of the healing time and bone-to-implant anchorage, the value of the quality and quantity of this dynamic biologic process, nor whether these values are stable or variable.

The purpose of the present study was to analyze the quantitative bone behavior histologically, histomorphometrically, with Alizarin Red S staining, via BIC, and via the dynamic osseointegration index (DOI) and to evaluate the qualitative bone response with a new bone quality index (BQI) during the biologic process of osseointegration.

Materials and Methods

Animal Study

This animal study protocol was approved (05/2018) by the Ethics Committee for Animal Research of the Veterinary School of the University of La Havana (Havana, Cuba). The ARRIVE Essential 10 information and Recommended Set for the best practices were rigorously considered.

A total of 12 unloaded implants (Premium Straight, Sweden & Martina) were placed in three sheep tibia (4 implants per tibia). Three months after insertion, histologic, histomorphometric, and SEM-EDX (scanning electron microscopy with energy-dispersive x-ray spectroscopy) analyses were performed Figs 1 and 2. Implants (3.3-mm diameter × 8.5-mm height) were selected according to site dimensions, and each was surgically placed with the micro-implant platform at the cortical crest.

After 90 days, the animals were sacrificed, and the tibia specimens were retrieved, trimmed, and immersed in 4% formaldehyde solution for 5 days. In a histology lab (Faculty of Odontology, La Havana University), all specimens were dehydrated (graded ethanol series) and embedded in Technovit 7200 VLC resin (Kulzer). Then, all specimens were reduced to 7-µm and 60-μm thickness using a microtome (Exakt, Apparatebau) and were stained with Stevenel’s blue and Alizarin Red S. The histologic specimens were analyzed under a transmission light microscope (Axio Imager M2, Zeiss). The digital images (×1.25 magnification) were recorded using a digital camera (Axiocam 503 Mono, Zeiss) and were analyzed with a digitizing pad (STU-540, Wacom) and histomorphometry software (Image-Pro Plus, version 4.5, Media Cybernetics). The BIC percentage was calculated as the ratio between the amount of implant linear surface firmly in contact with the mineralized bone matrix compared to the total implant surface.

Dynamic Osseointegration Index

The BIC histomorphometric data were used to evaluate osseointegration. Therefore, the BIC rate was achieved only at the end of the healing time. The DOI was
determined as the ratio between the BIC percentage and the number of days and was evaluated around the implant at 15, 30, 60, and 90 days to obtain more complementary information of the dynamic bone behavior during osseointegration.

**Bone Quality Index**

The BQI was determined as a percentage of the ratio between the calcium (Ca) and phosphorus (P) ions (Ca/P) and was evaluated at specific and reproducible sites for each chamber (1, 3, and 5; a chamber is the space/bone volume between two coils of the implant screw) in relation to the native bone. In each chamber, the following four zones were identified: the titanium-bone interface (zone A), the middle chamber–bone front (zone B), the bone–surgical threading interface (zone C), and native bone (zone D; used as a control). A total of 20 SEM-EDX spectrums (Fig 1) were recorded per chamber (5 SEM-EDX spectrums per zone), totaling 60 scans per implant. BQI detected the osseointegration process during bone apposition, the bone front advance, and the implant anchorage into the maxilla. Data from the SEM-EDX Ca/P ion content, differences between zones, and the derived BQI indexes were used to evaluate biologic osseointegration quality, bone behavior, and bone density around implants during the observed healing time (90 days) before loading.

**Results**

**Results at 15 Days**

Optical microscopic views at low magnification (×5) of all histologic samples showed bone contact with the coronal portion of the implant, while the middle and apical portions were included in large bone marrow cavities. At ×20 magnification, chambers 1, 3, and 5 showed initial bone formation with osteoid matrix deposition into all chambers around the implant surface. Multiple zones with different color intensities (Alizarin Red S staining) were detected. In the center of all chambers, higher color intensities (L* = 51.49; a* = 57.68; b* = 44.17) were observed compared to the bone-implant interface (L* = 97.61; a* = –12.38; b* = 40.74). In several zones, bone absence or a low level of BIC was recorded (BIC = 23.3% ± 3.9%; DOI = 1.55) (Table 1).

The SEM-EDX analysis of all chambers showed a low mineralized zone of bone/bone marrow, with very low Ca (0.75% ± 0.03%) and P (0.39% ± 0.01%) ions present.
in zone A. In zone B, partially mineralized newly formed bone was detected, including Ca (1.58% ± 0.10%) and P (0.84% ± 0.11%) ions. In zone C, higher mineralized bone values were seen, with Ca (2.19% ± 0.13%) and P (1.10% ± 0.04%) ions present. In zone D, homogeneous bone structures with highly mineralized areas were detected, with very high Ca (2.97% ± 0.30%) and P (1.43% ± 0.15%) ions (Figs 1b, 1c, 2b, and 2e; Table 2).

**Results at 30 Days**

Optical microscopic views at ×5 magnification showed new BIC in all histologic specimens evaluated higher in the crestal portion; however, the bone, when evaluated near the cortical portion, appears to be of poor consistency (Fig 2). At ×10 magnification, the histologic evaluation showed bone formation in higher regions, extending into chamber 1, with multiple zones of new bone in the center and spreading to the implant surface. Multiple zones with different color intensities (Alizarin Red S stain) of the bone were observed in chambers 3 and 5. A higher red intensity was seen in the center of the chamber (L* = 53.27; a* = 57.07; b* = 43.32) than near zone A (L* = 92.17; a* = –16.28; b* = 38.56). A high level of BIC was detected along the threading length (BIC = 77.3% ± 0.4%; DOI = 2.58) (Table 1).

In all chambers, SEM-EDX analysis showed mineralized bone and bone marrow. In zone A, low Ca (2.30% ± 0.12%) and P (1.17% ± 0.02%) ions were recorded. In zone B, dense mineralized newly formed bone was detected with Ca (4.87% ± 0.29%) and P (1.73% ± 0.17%) ions present. In zone D, high homogeneous bone mineralization was seen, with Ca (6.63% ± 0.37%) and P (3.33% ± 0.12%) ions present. In zone D, high amounts of Ca (8.90% ± 0.85%) and P (4.37% ± 0.40%) ions were recorded (Figs 1e, 1f, 2c, and 2f; Table 2).

**Results at 90 Days**

Histologic analysis showed tight bone contact to the middle and apical implant portions in all samples, and several bone cavities were observed in the coronal portion. At a higher magnification (×20), the histologic evaluation showed initial bone formation with osteoid matrix deposition into chamber 1, with multiple zones of low newly deposited bone near the implant surface. Multiple zones with different color intensities of the bone were observed in all chambers (L* = 64.46; a* = 36.56; b* = 42.39), with lower color intensity in the center of the chamber (L* = 81.05; a* = 46.97; b* = 45.23) than at the interface between native bone and surgical threading portion (L* = 93.19; a* = –11.46; b* = 38.23). A low, increasing percentage of BIC was detected in all specimens (BIC = 86.4% ± 0.6%; DOI = 0.9%) (Table 1).

SEM-EDX analysis showed a larger area of mineralized bone/bone marrow in all chambers, with very high Ca (2.30% ± 0.46%) and P (1.07% ± 0.05%) ions in zone A. In zone B, partially mineralized newly

---

**Table 1 BIC and DOI Evaluations at 15, 30, 60, and 90 Days**

<table>
<thead>
<tr>
<th></th>
<th>15 d</th>
<th>30 d</th>
<th>60 d</th>
<th>90 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIC, %</td>
<td>23.3 ± 3.9</td>
<td>77.3 ± 0.4</td>
<td>85.4 ± 0.3</td>
<td>86.4 ± 0.6</td>
</tr>
<tr>
<td>Change</td>
<td>–</td>
<td>+54%</td>
<td>+8.1%</td>
<td>+1%</td>
</tr>
<tr>
<td>DOI</td>
<td>1.55</td>
<td>2.58</td>
<td>1.42</td>
<td>0.96</td>
</tr>
<tr>
<td>Change</td>
<td>–</td>
<td>+66.4%</td>
<td>–44.7%</td>
<td>–32.4%</td>
</tr>
</tbody>
</table>

BIC = bone-to-implant contact; DOI = dynamic osseointegration index.

DOI was evaluated as the ratio between BIC percentage and the number of days. The index evaluates the bone growth percentage in the range of time useful to better define the value and speed of osseointegration as a dynamic biologic process.
formed bone was detected, with Ca (6.37% ± 1.75%) and P (3.27% ± 1.00%) ions present. In zone C, more mineralized bone was seen, with Ca (7.93% ± 2.60%) and P (3.90% ± 1.15%) ions present. Zone D presented homogeneous bone structures with highly mineralized areas, with many Ca (9.10% ± 3.02%) and P (4.37% ± 1.31%) ions present (Figs 1d, 1h, 2d, and 2g; Table 2).

**Discussion**

Only after implant placement can the osseointegration process begin. Therefore, clinical methodologies used to detect the removal torque...
value or the mechanical stability of the screw cannot be considered proper methods to evaluate the biologic process of osseointegration. Furthermore, Newesely and Davies hypothesized two distinct osseointegration processes related to the profilometric titanium surface characteristics. The first, termed “distance osteogenesis,” involves the new bone growth from native bone to the implant machined surface. The second, called “contact osteogenesis,” described the process when new bone grows in direct contact with the roughened implant surface. Recently, Zamparini et al showed low, moderate, or high bone quantities around implants after osseointegration, while Mastrangelo et al introduced the new DOI to evaluate the dynamic bone behavior during osseointegration of three different implant surfaces.

In the present study, 15 days after implant placement, histologic analysis showed an intimate contact between the bone and the implant surface only in the implant head area (chamber 1). The implant body (chamber 3) and apex (chamber 5) showed large empty cavities, with bone marrow at a distance from the surface. The Alizarin Red S staining showed different intensities, with very low red color in the wide bone layer near implant surface and intense red coloring in areas near native bone. After 30 days, the samples showed a bone density increase in all chambers. Moreover, irregular spots of bone deposition in implant chambers 1 and 3 were detected, as well as an increase in the red color intensity of the bone; alternatively, chamber 5 showed lower bone deposition values and less red color intensity of the bone. At 90 days, bone was adhering to the implant surfaces in chambers 1 and 3, with medium and large osteons detected, while a large part of undifferentiated bone was observed in chamber 5. In chambers 1 and 3, lower red color intensity was observed compared to high color intensity in chamber 5, confirming a high biologic bone activity in this area.

| Zone A | Mean (BQI, %) | 15 d | 25.35 ± 2.01 | 2.30 ± 0.12 | 25.91 ± 1.90 | 2.30 ± 0.46 | 26.25 ± 6.27 | 26.50 ± 6.27 |
| Zone B | Mean (BQI, %) | 30 d | 27.16 ± 3.15 | 1.17 ± 0.02 | 26.86 ± 3.15 | 1.07 ± 0.05 | 25.82 ± 6.49 | 25.82 ± 6.49 |
| Zone C | Mean (BQI, %) | 90 d | 53.58 ± 10.97 | 4.87 ± 0.29 | 55.29 ± 10.98 | 6.37 ± 1.75 | 71.07 ± 7.00 | 71.07 ± 7.00 |
| Zone D | Mean (BQI, %) | 15 d | 58.28 ± 1.52 | 1.73 ± 0.17 | 40.05 ± 12.36 | 3.27 ± 1.00 | 75.27 ± 9.92 | 75.27 ± 9.92 |

BQI = bone quality index; Ca = calcium; P = phosphorus; SEM-EDX scanning electron microscopy with energy-dispersive x-ray spectroscopy; zone A = titanium-bone interface; zone B = middle chamber-bone front; zone C = bone-surgical threading interface; zone D = native bone (control).

Values are presented as means ± SD of the specimens from chambers 1, 3, and 5. Ca and P values at 60 days were omitted for clarity of data, as they were very similar to 30-day findings.
observation period. Nonetheless, only 23.3% of BIC was recorded after 15 days, while the greatest BIC growth (77.3%) was recorded 30 days after implant placement, confirming that such a time frame is critical for the osseointegration process. Indeed, after 60 (85.4%) and 90 days (86.4%), bone apposition slowdown was observed. The DOI index confirmed a high osseointegration growth after the first 15 days (1.55), with a maximum increase seen after 30 days (2.58). At 60 and 90 days, the decreasing bone deposition showed that the osseointegration process is a variable and inconsistent progression.

After 15, 30, and 90 days, the SEM-EDX analysis and the Ca/P color mapping reconstruction showed a progressively increased average value in all samples from zone A toward zones B, C, and D (zone D was considered a control). In zone A, Ca and P percentages remained constant after the initial increase, and the BQI value always remained around 25%. BQI data showed a maturation degree and lower bone quality at the interface between the implant and the bone surface (zone A) compared to the other zones. At 15 and 30 days, zones B and C (except for P in zone B) in all chambers showed BQI values slightly over 50% and around 75%, respectively, confirming that a progressively higher degree of bone maturation occurs together with the osseointegration process. After 90 days, BQI values around 90% were detected in zone B (Ca = 71.01% ± 7.00%; P = 75.27% ± 9.92%) and zone C (Ca = 87.51% ± 7.60%; P = 90.02% ± 11.06%) and C, confirming that bone maturation and osseointegration undergo an acceleration in this phase.

By evaluating the Ca and P values obtained and the BQI scores achieved in the present study, the present authors were able to assess how the osseointegration process proceeds vertically and horizontally. In the first 15 days after implant placement, bone showed a high degree of horizontal growth, with new bone deposited directly on the implant surface, supporting increased “contact bone growth” during early osseointegration (EO). In EO, the implant surface seems to play a crucial role, related to the increased contact area due to the implant micro- and nano-roughness and the increased chemical-physical adhesion of the clot to the rough titanium surface. After 30 days, a larger bone-to-implant adhesion was detected, as confirmed by both BIC and DOI values. Active osseointegration (AO) was visualized with new bone deposited both inside the chamber and on the titanium surface. After 90 days, termed “later osseointegration” (LO), horizontal bone growth slowed down, achieving around 85% zonal BIC, whereas the growth curve abruptly decreased. High vertical bone growth was detected during EO in chamber 1 and low bone growth in chambers 3 and 5. This biologic process is seen at both 30 and 90 days, when mature and dense bone can be observed in chambers 1 and 3. During EO, a greater component of bone growth by contact was detected; during the AO and LO phases, the bone increased with a maximum “at distance” component (growth of native bone to the implant surface, observed later in the osseointegration process). EO and AO bone quality data proved that the first 30 days are essential for osseointegration, with high bone deposition and augmentation seen around the implant. After 90 days, the results showed an incomplete bone maturation process compared to native bone, with bone mineralization values around 25% (BQI) at the implant interface.

Conclusions

Further studies with a large number of implants, longer observation period, different titanium surfaces, and different implant site preparation techniques are needed to confirm the results of this pilot animal study. However, the obtained data showed three different phases (EO, AO, and LO) of the osseointegration process with a variable and inconsistent bone behavior. The quality and quantity of bone growth and deposition confirmed the first 30 days as the crucial phase of osseointegration. During this phase, the new bone was deposited higher, vertically, in contact with the implant surface, which was likely related to the clot adhesion onto titanium microroughness. Low “at distance” bone growth was observed around the screw, confirming the key role of the surface during the early phase of osseointegration. During the AO and LO phases, only horizontal “at distance” bone deposition and maturation were detected, from the native bone toward the implant screw.
Finally, 3 months after implant placement and before loading, the BQI confirmed that the bone maturation process cannot be considered complete.

Acknowledgments

The authors declare no conflicts of interest.

References