Microchemical and surface evaluation of canine tibial plateau leveling osteotomy plates

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Objective—To determine the microchemical and surface composition of tibial plateau leveling osteotomy (TPLO) plates before and after explantation.

Sample Population—Seven TPLO plates surgically removed from host dogs 6 to 54 months after implantation; 2 raw unpolished-and-unpassivated 316L TPLO plates; and 2 heat-treated, polished-and-passivated, and cleaned 316L TPLO plates.

Procedures—Samples were removed by use of standard techniques to ensure the plate surface was not damaged. Sample pieces were dissolved and analyzed by inductively coupled plasma–mass spectrometry (ICP-MS) to determine bulk elemental composition. Other sample pieces were investigated by use of scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), and x-ray photoelectron spectroscopy (XPS) for determination of sample morphology, near-surface elemental composition, and surface elemental composition, respectively. To investigate the possibility of corrosion in situ, some samples were removed, chemically corroded, and analyzed.

Results—ICP-MS confirmed that elemental composition of samples was consistent with 316L stainless steel. The SEM and EDS analyses revealed trace amounts of polishing materials and a nonuniform carbonaceous biofilm on < 1% of the surface area of samples removed from the host dogs. The XPS analysis indicated an increase in the chromium-to-iron ratio on passivated surfaces, with no difference between passivated samples before implantation and after explantation.

Conclusions and Clinical Relevance—Composition of the TPLO plates was consistent with 316L stainless steel. No chemical or topographic changes were detected in TPLO plates that had been implanted in dogs for up to 54 months. A small amount of biofilm was evident on the surface of 2 plates. (Am J Vet Res 2007;68:908–916)

Injury of the cranial cruciate ligament remains the most common cause of hind limb lameness in adult large-breed dogs. Popular breeds such as Labrador Retrievers and Rottweilers are commonly afflicted with gradual tearing of the cranial cruciate ligament. Return to function is best accomplished through surgical intervention. Numerous techniques have been described to repair stifle joints that have deficiencies of the cranial cruciate ligament. A procedure favored by many small animal surgeons is the TPLO. This procedure changes the mechanics of the joint whereby cranial translation is converted to caudal translation, the latter of which is then neutralized by the caudal cruciate ligament. To accomplish the procedure, an osteotomy of the proximal tibial metaphysis is performed, and the tibial plateau is then rotated to a new position and stabilized with a bone plate and screws. A commercially manufactured plate has commonly been used for the TPLO stabilization. In the past few years, a possible increase in osteosarcoma in dogs that had undergone a TPLO stabilization. In the past few years, a possible increase in osteosarcoma in dogs that had undergone a TPLO procedure was suggested in a report. Furthermore, issues have been raised regarding the quality of that commercially available TPLO plate (ie, it may not meet established specifications, especially standards for surgical implants). For example, investigators in 1 study reported that intra- and extracellular particulate debris were detected in tissues samples obtained adjacent to the site of TPLO plates and concluded that this debris and mononuclear cell infiltrates observed were probably the result of corrosion. The same group of investigators also implicated the commercially available TPLO plate as a cause of neoplasia in a dog.
An additional report also revealed an osteosarcoma in the proximal portion of the tibia at the site of a TPLO performed by use of the commercially available TPLO plate. Crevice corrosion has also been reported in 7 explanted TPLO plates, and the investigators in that study concluded that the cast implants were inferior to wrought implants because it appeared that surface irregularities and porosity served as initiation sites for observed corrosion defects.

Osteosarcomas related to implants have been associated with chronic low-grade inflammation, most commonly secondary to loosening of the implant, galvanic corrosion, and low-grade infection. Osteosarcoma in a dog associated with a wrought stainless-steel plate has also been reported; in that dog, there was no histologic evidence of implant loosening or infection.

In vivo, 316L stainless steel is capable of a small amount of corrosion, but this corrosion can be limited by proper passivation, implant fixation (to minimize fretting corrosion), and use of materials composed of similar metals (to avoid galvanic corrosion). If long-term implantation of the commercially available TPLO plate results in substantial corrosion, chronic inflammation could result in the development of bone tumors at or near the TPLO site. Therefore, the goal of the study reported here was to determine the microchemical and surface composition of commercially available TPLO plates, particularly as they relate to corrosion, before implantation and after explantation from host dogs.

**Materials and Methods**

**Sample population**—Three types of commercially available TPLO plates were used in the study reported here. Plates included 2 unpolished, unpassivated 316L stainless-steel blanks, 2 polished-and-passivated plates that were suitable for implantation, and 7 explanted specimens that were removed from host dogs between 6 and 54 months after implantation. Plates were removed from the host dogs during an exploratory arthroscopy performed because the dogs developed a new lameness on the previously operated limb with signs of pain isolated to the stifle joint, and it was suspected that they had late meniscal tears or that the plate may have been the cause of the new pain or lameness.

**Explantation of TPLO plates**—Removal of in situ TPLO plates was accomplished through a medial incision. Sharp dissection was used to expose the surface of each plate. A combination of sharp and blunt dissection was used to free each plate from soft tissues that encased it. Care was taken to ensure the surface of each plate was not damaged during removal. Screws were removed, and plates were lifted from the surface of the bone. Gross inspection of the soft tissues adjacent to the plate surfaces was completed, and the soft tissues were sutured by use of standard methods. After removal, each plate and the corresponding screws were gently washed with distilled water, dried with a towel, and placed into clean dry plastic containers until analyzed.

**Preparation of explanted samples**—Explanted samples were prepared for analysis. Prior to analysis, all samples were rinsed with deionized water for 30 seconds, rinsed in isopropyl alcohol for 30 seconds, and then dried under a stream of nitrogen gas until the surface appeared dry. To accommodate the various mounting geometries of the analysis instruments, TPLO plates were mechanically sectioned into pieces (approx 10 × 10 × 5 mm) by use of a rotary diamond saw.

**Optical microscopy**—Digital micrographs were obtained by use of a digital optical microscope equipped with a variable magnification objective (20× to 600× magnification), integral fiber-optic light source, and charge-coupled device camera. Digital images were saved as 640 × 480 JPG files.

**ICP-MS**—Analysis by use of ICP-MS provides information about the elemental composition of the bulk metal from which plates are constructed. Samples were weighed and then digested in aqua regia (HNO₃:HCl [1:4]). After digestion for 24 hours, samples were diluted with a weak nitric acid solution (HNO₃:H₂O[1:1,000]). Samples were then analyzed with a mass spectrometer fitted with a quadrupole mass filter. Each analysis represented the mean of 10 averaged measurements. To eliminate potential effects of interference from the sample matrix, 5 calibration standards were prepared from separate elemental standards to approximate the concentrations in 316L stainless steel; specifications for the calibration standards were as indicated in a guidance document. Aluminum was added to these calibration standards to ensure that, when necessary, it would be detected and quantified. These 5 standards were then used to construct the calibration curve used to determine concentrations of the samples. The 5 standards were analyzed immediately before and after the samples to ensure consistency in measurements.

**SEM and EDS**—Scanning electron micrographs were obtained on a scanning electron microscope fitted with a liquid nitrogen–cooled Princeton gamma tech EDS system and on a scanning electron microscope fitted with an in-lens annular detector and a thermal field-emitter source. All images were acquired by use of an accelerating voltage of 15 kV and a working distance of 10 mm. The EDS spectra were integrated for 60 or 100 seconds (scanning electron microscope fitted with the EDS system or the scanning electron microscope fitted with the in-lens annular detector and thermal field-emitter source, respectively) to achieve a high signal-to-noise ratio. Identification of the EDS peaks was determined by use of commercially available software. Use of EDS provided data on elemental analysis of stainless steel (density of 8 g/cm³) to a depth of approximately 3 µm by use of an accelerating voltage of 15 kV.

**XPS**—Data for XPS provided elemental analysis of the top few nanometers of a plate surface. The XPS data were obtained with a commercially available system by use of an Al kα anode source operating at 200 W. Detector pass energy was set at 80 eV, and datum points were collected at 0.5-eV intervals. Each spectrum represented the mean of three 180-second acquisitions. Base pressure in the analysis chamber was < 5 × 10⁻⁹ torr for all analyses. Calibration of the energy scale was accomplished for each sample by setting the carbon 1s peak...
electron orbital peak to 284.8 eV; the letter s (and also p, d, and f) refers to the spectroscopist designation for atomic subshells.

**Intentional corrosion**—To ensure that any corrosion on the explanted plates would be detected, 2 polished-and-passivated TPLO plates were intentionally corroded (oxidized) by exposure to hydrochloric acid for 30 minutes. These plates were prepared by immersion in a polytetrafluoroethylene-coated beaker that contained concentrated hydrochloric acid heated to 65°C. After exposure to the acid, the plates were thoroughly rinsed in deionized water and placed under a stream of nitrogen gas until dry. These plates were then

![Figure 1](image_url)

*Figure 1*—Photomicrographic views (A and B), SEM images (C and D), and EDS spectra (E and F) of a commercially available TPLO plate removed 12 months after implantation (A, C, and E) and following acid-induced corrosion after explantation (B, D, and F). Panels A, C, and E were obtained from the same locations as for panels B, D, and F, respectively. In panels A and B, the depression between the compression holes resulted from manipulation by the surgeon to contour the plate to the bone surface. Panels C and D are SEM images obtained from the depression between the compression holes. In panel D, notice the small corrosion-induced pits (circles). Panels E and F represent an EDS trace obtained at the center of the depression between the compression holes to reveal the content of carbon (C), Fe, Ni, Mo, Cr, Mn, and oxygen (O). Bars in panels A and B = 5 mm; bars in panels C and D = 20 μm.
analyzed by use of the same SEM and EDS procedures as described previously.

**Results**

The surface of the unpolished plates had a dull metallic matte appearance, which is in contrast to the mirror-like finish after polishing and passivation. An optical micrograph was obtained of a polished-and-passivated plate explanted after 12 months in situ (Figure 1). This plate, as well as all the other explanted plates, was quite similar in appearance to polished-and-passivated plates before implantation. At this qualitative level, the only substantial difference between explanted and unused plates was the change in plate morphology that resulted from manipulations performed by the implanting surgeon to conform the plate to the surface of the tibial metaphysis. These manipulations changed the overall shape of the TPLO plate to differing degrees and also introduced small concave indentations as a result of local compression caused by the metal used to bend the plate. Additionally, the surface of 2 of the 7 explanted plates used in the study had small (<1 mm²) spots of a reddish-brown film. These spots were only apparent near the compression holes on the convex surface of the plates. Use of SEM and elemental analysis by use of EDS confirmed that these films were composed primarily of carbon (Figure 2).

The ICP-MS was used to determine the elemental composition of the TPLO plates. To achieve representative quantitative results, we chose to completely dissolve 1-cm sections of the plates, dilute the resulting solution, and then perform ICP—MS analysis on aliquots of the diluted solution. Complete dissolution of the plate samples required 2 acid-digestion steps. Specifically, a small amount of undissolved gelatinous material remained after the initial 24-hour digestion in aqua regia. It was not possible to isolate the gelatinous material by filtration for direct analysis. Accordingly, 0.1 mL of 49% hydrofluoric acid was added to the original solution, and this treatment completely dissolved the remaining gelatinous material within 30 seconds and yielded a precipitate-free, homogeneous solution. Because this material was insoluble in aqua regia but dissolved readily in hydrofluoric acid, it was considered likely to have been a silica gel arising from the small amount of silicon contained in 316L stainless steel. Elemental percentages of Fe, Cr, Ni, Mo, Mn, and Al obtained by ICP-MS analysis were summarized (Table 1). Although Al is not a component of 316L stainless steel, we conducted an analysis to measure the Al content because it has been reported that Al can be a potentially problematic impurity in implant materials. We did not attempt to quantify the amount of carbon, silicon, phosphorus, and sulfur because they are trace elements in 316L stainless steel. All of the major elements comprising 316L stainless steel were found within the range specified in the guidance document, except for Ni. Nickel content was below the specified range by 3.0% and 3.6% in the polished and unpolished plates, respectively, and by a mean of 6.1% in the 7 explanted TPLO plates.

Although SEM provides high-resolution images of the microstructure of the surface of TPLO plates, it provides a limited field of view; thus, it was not possible to obtain an SEM image of the entire surface of the plates. Therefore, to obtain a characteristic representation of the surface morphology, a minimum of 10 images were obtained for each plate from a number of locations widely distributed over the surface of each plate. Even
at magnifications as high as 2,000X, the surfaces of similar plates were remarkably uniform in appearance.

Unpolished TPLO plates had a rough appearance in SEM micrographs. In contrast, polished-and-passivated plates had much smoother surfaces (Figure 3). The surface of polished plates was generally flat, but some irregular features (5 µm in diameter) were also observed. The EDS analysis indicated that flat regions of the surface were characterized by elements associated with 316L stainless steel. However, the small irregular features contained Al and oxygen.

Control experiments were performed to intentionally corrode small sections of the surface of several TPLO plates (unpolished and polished blanks and an explanted TPLO plate) by exposing them to concentrated hydrochloric acid for 30 minutes. A low-resolution optical micrograph was obtained for the explanted TPLO plate after acid-induced corrosion (Figure 1). At that low resolution, the surface appeared slightly more reflective than it did prior to corrosion, but there were no other obvious changes. However, at a higher resolution, substantial differences were apparent. Specifically, after acid-induced corrosion, an interconnected network of channels was apparent on the surface of the explanted plate, which represented selective corrosion at the grain boundaries of the stainless steel. Also, numerous small corrosion-induced pits were evident. The EDS data obtained from the intentionally corroded plate revealed the characteristic Fe 2p electron peak located at 712 eV, which corresponded to oxidized Fe (Fe²⁺ or Fe³⁺). However, in the polished-and-passivated (but not implanted) and explanted plates, Fe was barely detectable. This result was anticipated because the passivation process intentionally removes Fe until a Cr-rich grain boundary is formed. It is this repassivation process that differentiates stainless steel from mild steel and allows the surfaces of stainless steel implants to remain clinically inert.

Discussion

It has been speculated in the orthopedic veterinary community that there are problems associated with materials in the commercially available TPLO plate. These problems include Al in or on the surface of the TPLO plates; elemental composition of the stainless steel in the TPLO plate that is inconsistent with that of the standard cast stainless steel used in implants; and concerns that as a TPLO plate matures in vivo, it undergoes corrosive processes (pitting, fretting, or galvanic corrosion). Accordingly, the study reported here was conducted to address these 3 issues by analyzing...

### Table 1—Elemental composition of commercially available TPLO plates* and standards23 for cast 18 Cr–12.5 Ni–2.5 Mo stainless steel for surgical implants.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fe (%)</th>
<th>Cr (%)</th>
<th>Ni (%)</th>
<th>Mo (%)</th>
<th>Mn (%)</th>
<th>Al (%)</th>
</tr>
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<tbody>
<tr>
<td>Standard</td>
<td>61.65–72.00</td>
<td>17.00–19.00</td>
<td>11.00–14.00</td>
<td>2.00–3.00</td>
<td>0.00–2.00</td>
<td>0*</td>
</tr>
<tr>
<td>Unpolished-and-unpassivated (n = 2)</td>
<td>68.30</td>
<td>18.66</td>
<td>10.62</td>
<td>2.39</td>
<td>0.55</td>
<td>0*</td>
</tr>
<tr>
<td>Polished-and-passivated (n = 2)</td>
<td>68.27</td>
<td>18.58</td>
<td>10.68</td>
<td>2.38</td>
<td>0.58</td>
<td>0*</td>
</tr>
<tr>
<td>Explanted (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>67.19</td>
<td>18.15</td>
<td>11.91</td>
<td>2.60</td>
<td>0.83</td>
<td>0*</td>
</tr>
<tr>
<td>2</td>
<td>68.13</td>
<td>18.87</td>
<td>10.05</td>
<td>2.52</td>
<td>0.97</td>
<td>0*</td>
</tr>
<tr>
<td>3</td>
<td>67.70</td>
<td>19.93</td>
<td>10.33</td>
<td>2.62</td>
<td>1.01</td>
<td>0*</td>
</tr>
<tr>
<td>4</td>
<td>67.61</td>
<td>19.07</td>
<td>10.23</td>
<td>2.62</td>
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<td>0*</td>
</tr>
<tr>
<td>5</td>
<td>68.59</td>
<td>18.56</td>
<td>9.68</td>
<td>2.67</td>
<td>1.09</td>
<td>0*</td>
</tr>
<tr>
<td>6</td>
<td>67.45</td>
<td>19.03</td>
<td>10.34</td>
<td>3.13</td>
<td>1.09</td>
<td>0*</td>
</tr>
<tr>
<td>7</td>
<td>68.30</td>
<td>18.77</td>
<td>10.03</td>
<td>2.05</td>
<td>0.95</td>
<td>0*</td>
</tr>
</tbody>
</table>

Values reported represent the mean of 10 separate ICP-MS measurements, except for Explanted plates 6 and 7, which represent the mean of 5 ICP-MS measurements. All variations were < ± 0.1%. Because the percentage of each element was determined by a calibration curve created by use of known standards, the totals for each plate may not sum to exactly 100%. Values in parentheses represent the tolerance. *The amount of Al in the sample was less than the limit of detection for ICP-MS.
the materials and surface properties of TPLO plates. We used 3 types of plates in the study (unpassivated-and-unpolished TPLO 316L stainless-steel plates, passivated-and-polished plates intended for implantation, and passivated-and-polished plates that had been surgically removed from host animals 6 to 54 months after implantation).

Our strategy to determine the compositional structure of the plates involved chemical analysis for 3 depth scales. First, complete acid digestion and analysis by ICP-MS provided elemental analysis of the entire plate. Second, SEM and EDS provided the elemental composition of approximately the outer 3 µm of the plates. Third, XPS provided information about the elemental composition and oxidation state of approximately the outer 3 nm of the plate surface.

In addition to differences in depth resolution, XPS and the combination of SEM and EDS provided 2- and 1-µm spatial resolution in the plane of the plate surface, respectively. Our multiple-scale analysis approach did not reveal Al in the TPLO plates. The ICP-MS analysis indicated that there was no Al contained in the plates. Analysis by use of SEM and EDS indicated that there was some Al on the surface of the plates. This Al signal was accompanied by an oxygen signal. The Al and oxygen most likely were in the form of inert alumina (Al₂O₃) polishing particles that remained on the surface. These remaining polishing particles are much harder than stainless steel (hence, the reason they are used as a polishing medium), and therefore they can become embedded within the surface of a plate. Additional evidence that the particles were residual polishing medium was the fact that their size and shape were consistent with the polishing media used on TPLO plates. Furthermore, these particles were not found on the unpolished plates. It also appears that once these particles become embedded on the metal surface, they are not dislodged while the implant is in the host body (ie, the surface density of these particles was similar regardless of time until explantation).

The perception that the commercially available TPLO plates are constructed from an alloy that does not meet the standard for 316L stainless steel was addressed by the ICP-MS experiments. The ICP-MS analysis revealed that the elemental composition of the plate material conformed to the standard specifications, except for the percentage of Ni. The specification for Ni is between 11.00% and 14.00% with a tolerance of 0.15%.

Eight of the 9 polished-and-passivated plates used in the study contained Ni content lower than the specification standard of 11.00% (Table 1). Mean Ni content was 10.43% for all plates tested. However, this slightly lower percentage of Ni should not adversely affect corrosion resistance of the stainless steel. A reduction of 0.5% Ni will lower the pitting potential by only 0.001 V. The small decrease in Ni content should slightly affect the ratio of the ferrite phase to the austenitic phase. By use of a Schaeffler diagram, a 0.5% change in the Ni content will change the ferrite content from 4% to 6%.
The change in the amount of δ ferrite was determined by use of the Cr equivalent-to-Ni equivalent ratio calculated by use of the following equation for the Ni and Cr equivalents:

\[
\text{Cr equivalent} = \text{Cr} + (1.5 \times \text{silica}) + (1.4 \times \text{Mo}) + (\text{Nb} - (4.99 \times \text{Ni equivalent})) + (0.5 \times \text{Mn}) + (126 \times (\text{nitrogen} - 0.02)) + 2.77
\]

The small change in the percentage of possible δ ferrite could slightly alter the mechanical and magnetic properties but not the corrosion resistance.

To address concerns about the possibility that the commercially available TPLO plates corrode in situ, we intentionally corroded TPLO plates so that we could assess the effect of intentional corrosion on each of our analytic techniques. Direct comparison of samples with uncorroded and intentionally corroded surfaces provided an excellent means for assessing evidence of corrosion. Accordingly, small sections of the surface of unpolished-and-unpassivated, polished-and-passivated (but not implanted), and polished-and-passivated explanted plates were intentionally corroded by exposing them to hydrochloric acid. Except for slight differences in reflectivity, the plates did not differ substantially in appearance before or after intentional corrosion. In contrast to these results, it was easy to distinguish differences in the appearance of the surfaces by use of high-resolution SEM. For example, cracks and pits were evident on the intentionally corroded surface but not on the explanted plate that was not intentionally corroded (Figure 1).

Figure 4—Graphs of XPS analysis of TPLO plates for an unpolished-and-unpassivated plate (black line) and a polished-and-passivated plate (red line; A) and high-resolution XPS scans of the Fe 2p (B) and Cr 2p (C) regions. O = Oxygen. s and p = Spectroscopist designation for atomic subshells. BE = Binding energy. 1/2 and 3/2 = Different electron spin states. KVv and KLL = Designations for the atomic subshells involved in the Auger process.
Examination of micrographs also revealed obvious differences for the explanted plate. The EDS is particularly useful for distinguishing between corroded and uncorroded plates. Specifically, a high oxygen content, which is characteristic of corrosion products of stainless steel, was found in the EDS spectrum of the intentionally corroded explanted plate but not in the spectrum of the plate that was not exposed to hydrochloric acid (Figure 1).

In 2 explanted plates, a reddish-brown film was detected near the compression holes (Figure 2). Without the benefit of a high-magnification SEM or EDS analysis, these films appeared similar in color and structure to Fe oxide, a corrosion product of stainless steel. However, analysis of the EDS spectra indicated a large amount of carbon in this film but not in the nearby film-free region. Moreover, white areas evident in the regions were believed to arise from electron charging, which is characteristic of an insulating region of the surface, such as a biofilm.37 Analysis of these results indicated that the structure was a biofilm rather than Fe oxide (detection of Fe oxide would indicate crevice or fretting corrosion). The biofilms were quite similar in appearance to areas interpreted as arising from corrosion in another study of TPLO plates. Similarities in the analysis provided here and in that study2 include the results of the EDS analysis, including high carbon content, and evidence of electronic charging in the SEM micrographs.

Differences in contrast in SEM images that may have been interpreted as corroded regions of the plate represented topographic features that affected light reflectivity of the microscope (Figure 1). Additionally, there are regions in the SEM images that are indicative of differences in electron scattering, which EDS confirmed to be compositionally identical. One would expect an increase in the amount of oxygen signal in the EDS spectrum of a corroded area.

We have also found that XPS can distinguish differences between raw unpolished plates and those that have been heat treated, polished, passivated, and cleaned. However, its extreme surface sensitivity makes it difficult to detect evidence of corrosion in the 7 explanted TPLO plates investigated in our study.

We were unable to find evidence of corrosion in the 7 explanted TPLO plates investigated in our study.

References

20. Rose BW, Novo RE, Olson EJ. Osteosarcoma at the site of a triple


