# TISSUE REACTION TO IMPLANTS OF DIFFERENT METALS: A STUDY USING GUIDE WIRES IN CANNULATED SCREWS

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#### Abstract

#### Introduction

Cannulated screws, along with guide wires, are typically used for surgical fracture treatment in cancellous bone. Breakage or bending deformation of the guide wire is a clinical concern. Mechanically superior guide wires made of Co-Cr alloys such as MP35N and L605 may reduce the occurrence of mechanical failures when used in combination with conventional (316L stainless steel) cannulated screws. However the possibility of galvanic or crevice corrosion and adverse tissue reaction, exists when using dissimilar materials, particularly in the event that a guide wire breaks, and remains in situ. Therefore, we designed an experiment to determine the tissue reaction to such an in vivo environment. Implant devices were designed to replicate a clinical situation where dissimilar metals can form a galvanic couple. Histological and SEM analyses were used to evaluate tissue response and corrosion of the implants. In this experiment, no adverse in vivo effects were detected from the use of dissimilar materials in a model of a broken guide wire in a cannulated screw.

Keywords: Animal model, Bone, Cobalt alloy, Stainless steel, Corrosion

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Telephone Number: +41 81 414 23 21, 414 22 11 FAX Number: +41 81 414 22 88 E-mail: declan.devine@aofoundation.org Cannulated screws, together with guide wires, are frequently used in orthopaedic surgery primarily for the repair of fractures in cancellous regions. Cancellous screws are usually made either of titanium or stainless steel with corresponding guide wires made of the same material. The guide wires aid in the correct placement of the cannulated screws and are removed after screw placement (Rüedi *et al.*, 2007). However, it is possible for a wire to break off or bend at the tip of the screw (Mishra *et al.*, 2002; Upadhyay *et al.*, 2004). This problem can occur as a result of the bending of the guide wire at insertion, such as if it hits a hard cortex. Breakage may then occur subsequent to using cannulated instruments along the bent guide wire, or on attempts to remove the bent guide wire.

In some situations only part of the guide wire can be removed or the remaining part of the guide wire may remain in contact with the screw. In both cases the wire fragment cannot be surgically removed without increased iatrogenic tissue trauma to the patient. A broken instrument which cannot be removed becomes an implant and must fulfil the biological requirements of implantable material. In order to limit the occurrence of guide wire bending and breakage and to optimize handling performance of guide wires, it is desirable to create a stiffer and stronger guide wire. One option to increase the mechanical properties of the guide wire is to increase the diameter of the guide wire. The consequence of this is that all cannulated instruments and implants must also be changed in order to accommodate a larger guide wire. This is undesirable, particularly because the current design and dimensions of cannulated devices are known to have clinical relevance and value (Rüedi et al., 2007). Furthermore, a larger diameter guide wire would require a larger hole in the screwdriver and the screwdriver tip may become critically weak. An alternative option is to use a material with improved mechanical properties in comparison to stainless steel. Although with either option the guide wires could still bend or break, the likelihood of that occurring would be greatly reduced.

A Nickel-Cobalt-Chromium-Molybdenum based biocompatible alloy called MP35N has a unique combination of properties which have enabled it to be a well established implant material (Younkin, 1974). A Cobalt-Nickel-Chromium-Tungsten based alloy, L605, has similar properties to MP35N (Poncin *et al.*, 2004). Both these alloys are generally recommended for applications where a combination of high strength, high modulus and good corrosion resistance are required (Escalas *et al.*, 1975; Williams, 1981; Marti, 2000; Hanawa, 2002; Poncin *et al.*, 2004). Both materials have





**Figure 1:** The design of the implants to test crevice and/or galvanic corrosion. The implant was designed to replicate situations where an alloy guide wire breaks within a stainless steel cannulated screw. Crevice corrosion was analysed using varying crevice dimensions. No fretting was involved in this model.

mechanical properties superior to those of conventional stainless steel guide wires and thus the occurrence of breakages should decrease if these materials were utilized as guide wires.

The purpose of this study was to determine the biological response to these mechanically superior metals (MP35N and L605), when the material is coupled with stainless steel in a non-loaded situation. This would simulate a situation where the guide wire bends and breaks and cannot be removed leaving a condition of contact between the L605 and MP35N alloys and a stainless steel cannulated screw *in vivo*.

#### **Materials and Methods**

#### Implants

Custom-made implants were developed as illustrated in Fig. 1. The overall length of the implant was 24 mm. In all cases the screw was 316L stainless steel while the 1.7 mm diameter guide wire (centre component in Fig. 1) was interchanged between 316L (control), MP35N and L605. In the threaded region, the guide wire was press fit within the screw. A window (indicated by the white lines in Fig. 1) was cut into the cannulated screw from just within the press fit zone to the distal end of the screw. The cannulated part of the screw had a conical shape. This construct was considered to imitate a cannulated screw with a broken guide wire remaining *in situ*, which would simulate a situation of critical crevice and/or galvanic corrosion of dissimilar metals in a mechanically stable condition.

#### Animal model

This study was performed in an approved laboratory (ISO 9001:2000 certification) in accordance with the Swiss Animal Protection Law. Sixteen female Swiss mountain sheep, 2-4 years old, were used. These sheep were purposebred for research at the AO Research Institute and as such had similar genetics and were of similar size and weight. Sheep were chosen to be the animal model as the mechanical environment (limb loading) and response to biomaterials is comparable to humans (Pearce *et al.*, 2007). Furthermore, their bone size enables the use of the same instruments and implants as used for human subjects receiving clinical treatment. This was considered to be



Figure 2: An explanted bone core including the implant.

particularly important for this study, where the surface area of the implant may be expected to influence corrosion.

A bilateral model was used and all test materials were applied to each sheep. One of each of the three test combinations were implanted into the distal part of both femora. Screws with MP35N guide wires and the control screws containing 316L stainless steel guide wires were implanted into the left tibiae, and screws with L605 guide wires and control combinations were implanted into the right tibiae. The order of placement of each material was rotated to avoid a location bias. A total of 48 combinations of MP35N or L605 guide wires with 316L stainless steel screws and 64 combinations of 316L stainless steel guide wires and 316L stainless steel screws were implanted in the distal femur and proximal tibia of sixteen adult Swiss mountain female sheep. Two time points, one month and six months were used in this study, with eight sheep used for each time point.

The implants from the right femur were used for a separate, parallel investigation (results not presented). Implants from the left femur were evaluated using non-decalcified, plastic embedded histology and test items from the tibia were evaluated after removing the bone core, using scanning electron microscopy (SEM).

#### Surgery

Before surgery, the animals were sedated with intravenous injections of diazepam (Valium ®) 0.3 mg/kg and butorphanol (Morphasol®) 0.08 mg/kg. General anesthesia was induced with thiopental (Penthotal®) 4-8 mg/kg IV and maintained with isoflurane (approx 2% in oxygen: oxygen flow rate was approx 0.5 L/min). Three stab incisions on the medial aspect of the medial condyle of the right femur, and two stab incisions on the medial aspect of the proximal part of the right tibia were created. Through these incisions, threaded holes were drilled into cancellous tissue in each bone using a drill and corresponding tap. To ensure reproducibility of the screw placement in cancellous bone, a custom made jig was used during surgery. The test items were implanted bilaterally using identical procedures for the left leg. Postoperative pain relief was achieved by intramuscular injections of buprenorphine (Temgesic®) 0.1 mg/kg 3 times daily for 2 days and subcutaneous injections of carprofen (Rimadyl®) 4mg/kg once daily for 5 days.



Postoperative monitoring included general condition, weight bearing, wound healing and signs of inflammation. Animals were humanely euthanized by lethal overdose injection using pentobarbital, (Vetanarcol® 60 mg/kg) IV at the appropriate endpoint of the experiment. The sheep were initially sedated and removed from the group prior to euthanasia.

Post mortem, the devices implanted into the tibia and femurs were cored out in combination with surrounding bone using a custom made coring device, which ensured a bone collar of at least 3 mm around the implant (Fig. 2).

## Scanning electron microscopy (SEM)

The bone cores and isolated screws were placed in 100% ethanol until required. Excessive tissue was removed from the screws using a cleaning protocol developed for the study. Briefly, the samples were placed in a 2% solution of Proteinase K in phosphate buffer (PB) with a chlorine content of <0.025%, for 72 hours. This was followed by rinsing three times in distilled water, with a five minute ultrasonic cycle in the third rinse. The samples were then stored overnight in a nonionic surfactant (Triton-X 1% solution) and ultra sonicated the following morning for five minutes. Triton-X was removed by rinsing the screws three times in distilled water. Finally the screws were rinsed in acetone and placed in an oven at 40°C until dry.

The cleaned screws were examined using a Hitachi S4700 field emission scanning electron microscope (FESEM) (Hitachi High-Technologies Corporation, Tokyo, Japan) fitted with an Autrata yttrium aluminium garnet (YAG) back scattered electron (BSE) scintillator type detector. The images were taken in both secondary electron (SE) and BSE mode, with accelerating voltages of 5 kV or 10 kV. An emission current of 40µA and 20µA was used with the 5 kV and 10 kV accelerating voltages respectively. A working distance of 14-15 mm was used. Screws from each group were thoroughly examined to determine if visible corrosion had occurred. After this initial analysis, the guide wires were removed by forcing them out of the screw using a fresh guide wire. Images of the underside of the guide wires and the corresponding location on representative screws from each test group (three per group) were recorded in defined regions of interest. Additionally, images of non-implanted screws were taken to act as controls for each group at each location. Five images were recorded for each guide wire and screw starting 2 mm from the start of the window (position number one). The next three images were taken 2.5 mm beyond the previous one. The final image (position number five) was recorded at the end of the guide wire. Images were taken at a nominal magnification of 1000x. Additional images were taken at different magnifications depending on the artefact under investigation.

# Histology

The samples were fixed in 70% ethanol, dehydrated in an ascending series of alcohol solutions and immersed in xylene prior to embedding in methyl methacrylate. Sections 200  $\mu$ m think were prepared using a Leica 1600 circular saw (Leica AG, Glattbrugg, Switzerland). Using a cabinet X-ray system (Faxitron X-ray Corp., Lincolnshire, IL, USA, model #43885A), radiographs of each section were prepared. From these microradiographs three sections were chosen for further processing. The first location was 2 mm from the start of the window (position A). Two additional sections were selected at one third and two thirds of the distance to the guide wire tip from the first section (positions B and C, respectively). These sections were glued with cyanoacrylate to plexiglass, ground and polished to a thickness of 60-100 µm using an Exact Micro Grinding System (Mederex, Bath, UK). Sections were surface stained with Giemsa-Eosin for analysis. The sections were semi-quantitatively scored using the scoring system shown in Table 1 in defined regions of interest (ROI). The ROI were in front of the guide wire (window) and the crevice between the guide wire and the screw (gap).

Section locations were analyzed independently as the cellular response was likely influenced by the gap size, and was not constant between locations due to the conical shape of the cannulated screw. Each section was viewed at a magnification of 2.5x and an overview image of the section was recorded. From this image the distance from the centre of the guide wire to the nearest bone tissue perpendicular to the guide wire was measured. Additionally, each section was examined at 20x magnification to determine the cellular response to the screw/guide wire combination. All scoring was performed by a blinded observer.

# Statistical analysis

A non-parametric analysis for repeated measures (Friedman test) was used to compare histological score data for each parameter. Post-hoc analysis was performed using a Wilcoxon test. Significant differences were reported at  $p \le 0.05$ , trends were reported at  $p \le 0.09$ .

## Results

Postoperatively all animals tolerated the surgery well and recovered uneventfully, without evidence of lameness. One animal was removed from the study due to the incorrect placement of one screw. This animal was replaced in the study with a reserve animal. All wounds healed without complication.

## Scanning electron microscopy

Evaluation of the explanted screws after cleaning, demonstrated the removal of most of the tissue without the generation of noteworthy artefacts. Remaining tissue artefacts were present on the 316L stainless steel guide wire after one month implantation. It was noted that at an accelerating voltage of 5 kV the dark segments (low density material interrupted as tissue) were more apparent than at 10 kV. This would indicate that a thin layer of tissue was present, as the higher energy electrons easily pass through the low density material. Insertion marks caused by mechanical damage from the manufacturing of the implants were also visible (Fig. 3). Machining artefacts were visible perpendicular to the insertion marks.



Additionally, metal artefacts which were residual from the manufacturing process such as small burrs of metal protruding from the surface were present on the guide wire surface, collectively there metal particles were referred to as 'protrusions'. These protrusions were obviously on the surface and subsequently were not corrosion. Insertion marks did not occur on all guide wires. Furthermore, similar artefacts were seen on the fresh non implanted screws and guide wires when examined under SEM.

After one month implantation, the guide wires consisting of MP35N showed similar surface characteristics to those consisting of 316L stainless steel, with the exception of insertion marks, which were much less evident on MP35N implants. Tissue and protrusions were present to varying degrees on the surface of the guide wires. However, tissue formation was not always as clearly evident and thus secondary electron and back scattered electron images of the same areas were compared to determine if changes in density occurred. This method was used to clarify the presence of tissue. The dark segments visible in BSE indicate that the material had a lower density compared to the metal and it can therefore be concluded that these segments are tissue and not corrosion.

The L605 guide wires after one month had similar characteristics to those described for 316L stainless steel guide wires. Briefly, insertion marks, mechanical damage and machine marks were present to varying degrees. Protrusions and tissue residue were present on the surface of the L605 guide wires.

After six months implantation, the surfaces of the guide wires did not appear to have altered considerably when compared to the one month group. Again artefacts were present on the surface which warranted further investigation for all guide wire types. Some artefacts were suspected of been caused by corrosion. However after further magnification and evaluations with BSE they were determined to be a result of tissue adherence (Fig. 4). Additionally, isolated examples of surface defects of undetermined origin were observed. However, these defects occurred sporadically in all treatments for both time periods and evidence of tissue at the bottom of these defects was common. An example of this is shown in Fig. 5 which occurred in L605 guide wire at position number three.

# Histology

At the one month time-point, no significant difference was detected between any of the materials for all parameters evaluated. For the six month time period, the 316L stainless steel guide wire and screw combination had less bone tissue in the gap in position number B, but this was not significant (p=0.086). No differences were seen in all other parameters at the six month time period.

In general at the one month time period, the major change to the bone architecture was created by the hole drilled for placement of the implants which was still clearly evident (Figure 6a). Minor new bone growth or remodelling was detected. Bone in-growth into the space between the screw and guide wire was limited to small amounts observed in sections from position C. Connective tissue was consistently present in the gap for all implant



**Figure 3:** Images taken of the same guide wire (316L) after 1 month implantation at position number three using different magnifications. A tissue, B insertion marks, C machining marks, D protrusions.

material types. Importantly, at the one month time-point, there was no difference in cell reaction between groups.

At the six month time-point, remodelling of the drill hole was evident (Figure 6b), and newly formed bone was visible in the region in front of the window. In addition, bone growth into the gap between the guide wire and screw indicated that it was a tolerable environment for new bone formation.

Particles of opaque material were commonly observed on sections in the six month time period as shown in Fig. 7. These particles, were presumed to be of metallic origin, were not associated with a cellular reaction and no statistical differences in the presence of these particles could be detected between groups. The possibility of these particles being artefacts of sectioning could not be excluded.

## Discussion

The current study did not find evidence of an *in vivo* cellular response associated with the use of cobalt-chrome (Co-Cr) alloys with 316L stainless steel in a cannulated screwguide wire combination. The model chosen was designed to test galvanic/crevice corrosion and was developed to simulate 'the worst case scenario' in terms of the geometry,





**Figure 4:** L605 after 6 months implantation using secondary electron analysis (left) and back scattered electron analysis (right). After magnification and analysis with BSE, particles suspected of being caused by corrosion were found to be a result of tissue adherence.



**Figure 5:** L605 after 6 months implantation. In isolated cases, surface defects of undetermined origin were detected. These defects were sporadic and no significant difference was observed between groups.



**Figure 6:** a) Section C, 316L stainless steel guide wire after one month implantation showing limited bone re-growth into the drill hole created for placement of the implant; b) ) Section C,, L605 Co-Cr alloy guide wire after 6 month implantation showing bone formation in front of the window and in the gap.





**Figure 7:** Left: Particles present after six months implantation of the L605 guide wire combination, no significant adverse cellular response was observed; Right: Particles (arrows) present after six months implantation of the 316L stainless steel guide wire combination, no significant adverse cellular response was observed.

**Table 1**: Histological scoring system for PMMA sections.

# 1) New bone formation in front of the window (@ 2.5 Obj.)

	<ul> <li>Bone contact</li> <li>No bone contact, fibrous connecting tissue, (thickness &lt; 150 μm)</li> <li>No bone contact, fibrous connecting tissue (thickness between 150 – 500 μm)</li> <li>No bone contact, fibrous connecting tissue (thickness &gt; 500 μm)</li> </ul>	3 2 1 0
2)	Inflammatory cellular infiltration in front of the window (@ 20 Obj.)	
	Region of interest (average of measurements at either side of the window, Figure 3)	
	<ul> <li>No difference from normal tissue, no presence of inflammatory cells</li> <li>Presence of a few lymphocytes, macrophages, multinucleated giant cells, eosinophils and neutrophils (&lt;20/ FOV)</li> </ul>	0
	<ul> <li>Presence of several lymphocytes, macrophages, multinucleated giant cells, eosinophils and neutrophils (&gt;20/ &lt;50 FOV)</li> <li>Descence of lower of l</li></ul>	2
	<ul> <li>Presence of large numbers of symphocytes, macrophages, multinucleated grant cens, eosinophils and neutrophils (&gt;50/ &lt;100 FOV)</li> <li>Severe cellular infiltrate (&gt;100/ FOV) and/or tissue necrosis.</li> </ul>	3 4
3)	<ul> <li>Particles in front of the window (@ 20 Obj.)</li> <li>Zero presence of particles</li> <li>Presence of 1-5 particles</li> <li>Presence of &gt;5 particles</li> </ul>	0 1 2
4)	New bone formation in the gap (@ 2.5 Obj.)	
	<ul> <li>Bone area &gt; 75% of gap space</li> <li>Bone area between 50 - 75% of gap space</li> <li>Bone area between 25 - 50% of gap space</li> <li>Bone area &lt; 25% of gap space</li> <li>No bone in the gap</li> </ul>	4 3 2 1 0
5)	Inflammatory cellular infiltration in the gap (@ 20 Obj.)	
	<ul> <li>No difference from normal tissue, no presence of inflammatory cells</li> <li>Presence of a few lymphocytes, macrophages, multinucleated giant cells, eosinophils</li> </ul>	0
	<ul> <li>Presence of several lymphocytes, macrophages, multinucleated giant cells, eosinophils and neutrophils (&gt;20/ &lt;50 FOV)</li> </ul>	1
	<ul> <li>Presence of large numbers of lymphocytes, macrophages, multinucleated giant cells, eosinophils and neutrophils (&gt;50/&lt;100 FOV)</li> <li>Severe cellular infiltrate (&gt;100/ FOV) and/or tissue necrosis.</li> </ul>	3 4



in that the space between guide wire and the parent screw was gradually increased such that all possible material separations were present within the range in which corrosion may be expected. In addition, a window was created along the screw to optimise the exposure of the screw/guide wire implant to body fluids. The use of an inert screw (platinum or gold coated polymer) with the chrome cobalt alloys was considered in an attempt to have a positive control; however this scenario is remote in the clinical situation.

On implantation into the in vivo environment, material is initially in contact with extracellular body fluids such as blood and interstitial fluid. Body fluids contain 0.9% sodium chloride (the same as sea water) and other salts, amino acids and proteins that tend to accelerate corrosion as they can act as an electrolyte. Body fluids are naturally buffered and accordingly, their pH only changes minimally. The pH of normal blood and interstitial fluid is 7.35–7.45. However, the pH decreases to about 5.2 in hard tissue due to implantation, and recovers to 7.4 within 2 weeks (Hanawa, 1999; Hanawa, 2004). Metallic materials, in their solid state, do not show toxicity in vivo, but some dissolved metal ions, corrosion products, and wear debris may show toxicity when they combine with molecules and cells (Hanawa, 2002). Taking all these into consideration and in agreement with the conclusions of Rostroker (1978) and Kocijan and Milosev (2003), it is noted that in vitro studies alone are not definitive for the analysis of metal corrosion in vivo.

The present study utilized stainless steel cannulated screws. The performance of titanium implants when combined with the cobalt-chrome alloys cannot be predicted from this work. However, Marti (2000) states that galvanic corrosion is not observed when cobalt based alloys are in contact with titanium implants. This is due to the non conductive passive oxide layer which forms on titanium (Marti, 2000).

Stainless steel is one of the most popular biomaterials for internal fixation because of a favourable combination of mechanical properties, biocompatibility, corrosion resistance, and cost effectiveness when compared to other metallic implant materials (Disegi and Eschbach, 2000). However, it is also known that crevice and fretting corrosion can occur on bone plates and screws made of stainless steel, especially in the areas of contact between screw heads and plate-hole countersinks (Brown and Simpson, 1981). Shih et al. (2005) investigated the galvanic corrosion of 316L stainless steel wires with a mechanical indentation which locally altered the metals grain size. It was found that galvanic corrosion occurred between the different grain sizes both in vitro and in vivo. Therefore, it may be conceivable that some corrosion was occurring in the present study in the control group, but from the results observed, it appears that the use of the chrome-cobalt alloys does not exacerbate any corrosion occurring as a result of the implant geometry. Indeed, contrary to our expectations, the amount of new bone tissue in the gap (Section B) was decreased (trend) in the control implants compared to the test implants. Should this trend represent a real treatment effect the aetiology may include a smoother surface on the stainless steel guide wires

compared with the chrome cobalt alloys as surface roughness is know to affect cell adhesion (Hayes *et al.*, 2007) or possibly increased crevice corrosion in the control versus test implants.

Cobalt based alloys have been used in the manufacturing of orthopaedic surgical implants since the 1970s (Williams, 1981). Reasons for their acceptance as orthopaedic implants include excellent mechanical properties, corrosion resistance and biological tolerance. These metals were also considered among the best in terms of being biologically tolerated metals. Experimental data correlated ion migration with fibrous reaction around the implants and showed this effect to be minimal around Co-Cr-Mo, Co-Cr-Ni and Co-Cr-Ni-Mo in experimental animal implantation and suggested that Co-Cr alloys were well suited as skeletal implants from a biocompatibility point of view (Escalas et al., 1975). Younkin (1974) also found that the tissue from rabbits containing MP35N implants had a lower incidence of inflammatory changes than the tissue implanted with 304 stainless steel. It was also reported that the open crevice corrosion and stress cracking corrosion resistance of MP35N was superior to that of 316L stainless steel. It was concluded that the compatibility of MP35N was no different to that exhibited by 316L stainless samples and that it would not be unreasonable to conclude that MP35N would at least be equivalent to existing 316L stainless steel implants.

In the literature the results of coupling Co-Cr alloys and stainless steel is inconsistent. Younkin (1975) states that MP35N was extremely noble and caused galvanic corrosion of 316L stainless steel in seawater tests. Additionally, Marti (2000) states that Co-based alloys in contact with stainless steel are renowned for their corrosive attacks on steel. Therefore to avoid corrosive problems Co-based alloys should not be mixed with stainless steel implants. However, Reclaru et al. (2002) evaluated the galvanic current of a Co-Cr/REX 734 steel couple by direct measurement and by prediction theory. It was concluded that there was no appreciable risk for crevice corrosion caused or amplified by the galvanic coupling (Reclaru et al., 2002). In the study presented here no major signs of corrosion were visible using SEM. Although some localized artefacts were observed that could have indicated minor corrosion, the incidence of these artefacts was sporadic and did not vary considerably between test groups.

The particles observed on histological evaluation could potentially be of concern, however, the fact that no significant difference was detected between groups suggest that their presence does not indicate that the use of Co-Cr alloys as guide wires would increase the presence of particle formation *in vivo* when compared to 316L stainless steel. Furthermore, the absence of an associated cellular reaction around the particles support the possibility that these particles were created as an artefact of either the model used, or the analysis methods (i.e., while cutting and grinding the sections).

When metallic implants are placed *in vivo*, they initially undergo a passivation phase when ions are released. This passivation phase is essentially active corrosion of the surface. Hanawa (2004) reported that the oxide layer of



316L stainless steel not contain Nickel (Ni). In addition, Ornberg et al. (2007) reports that Co-Cr-Ni-Mo alloys preferentially release Ni ions during passivation. Therefore, during this passivation phase Ni is released from the surface of these materials into the surrounding tissue. In the histological sections from the one month time point, a cellular reaction to the initial passivation of the metal, which is essentially active corrosion of the surface, may have contributed to the lack of bone in front of the window. In this study, the percent Ni in the implants used in this study were 9 to 11%, 13 to 16% and 33 to 37% for the materials L605, 316L and MP35N respectively. Even though the Ni content of MP35N is at least double that of the other materials used in this study, it is considerably less than the Ni content of the Ni-Cr alloys tested by Brune (1986). Here Ni contents of up to 84% were used, and in the analysis of these results it was reported that in dental applications, the amount of Ni released from a Ni-Cr alloy containing 75% Ni in static conditions, released an order of magnitude less Ni than the daily dietary intake of the "standard man" (Brune, 1986). However, at the 6 months time point bone ingrowth into this area was evident. This indicated that the passivation phase of the implants used in this study was less than 6 months. This is in agreement with findings of Okazaki et al. (2004) who reported that the Ni concentration in tissue started to decrease at 6 weeks for 316L stainless steel implants in the tibia of rats.

Scanning electron microscopy analysis of sample guide wires prior to implantation indicate that numerous irregularities associated with manufacturing were present in each test group. Additionally, the insertion of the guide wires likely caused further surface changes that could result in the formation of metal particles on the surface of the guide wire or increased the susceptibility to corrosion and influenced the variability between samples (Disegi and Eschbach, 2000). Therefore, it is possible that responses observed experimentally may be associated with factors unrelated to the use of Co-Cr alloys in combination with stainless steel.

The intimacy of bone contact with the implants at six months suggest that despite the use of dissimilar materials in this cannulated screw and guide wire construct, the local environment provided stable implant anchorage and acceptable biotolerance.

## Conclusion

Despite the comprehensive evaluation of tissue from the animals in this study, we could not detect an adverse *in vivo* effect of using dissimilar materials (Co-Cr alloy with 316L stainless steel) compared with 316L stainless steel alone in a model evaluating galvanic/crevice corrosion of a broken guide wire in a stainless steel cannulated screw. This model did not study the effects of fretting corrosion.

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# **Discussion with reviewers**

**T. Hanawa**: Do you feel that 6 months *in vivo* is sufficient time over which to evaluate your hypothesis?

Authors: Yes. Okazaki *et al.* (2004) reports that the Ni concentration in tissue starts to decrease at 6 weeks for 316L stainless steel implants in the tibia of rats. Therefore the time points chosen should be sufficient to determine both pre- and post-passivation of the oxide layers. In addition, the international ISO standard 10993-6 - Biological evaluation of medical devices – part 6: *Tests for Local Effects After Implantation*, recommends the use of 26 weeks (6 months) as a time point for long-term implantation in bone.

