Pferdeheilkunde 27 (2011) 6 (November/Dezember) 652-663

Repeat cycles of drilling equine cortical bone and cleaning and sterilization change the surface characteristics of untreated and electrochemically polished surgical drill bits

Anna-Maria Henkel[1], Annemarie Langsteiner[1], Paul Linhardt[2] and Theresia Licka[1]

Abteilung Orthopädie und Chirurgie, Pferdeklinik, Department für Kleintiere und Pferde, Veterinärmedizinische Universität[1] und Institut für Chemische Technologien und Analytik, Technische Universität[2], Wien

Summary

We compared roughness, porosities, and corrosion of commercially available untreated and electropolished bone drills before and after use for drilling equine cortical bone. Material: Two-fluted surgical drills (steel classification AISI 440A). Electropolishing was carried out in HE 310 for two minutes in randomly selected drill bits. Drills were subsequently used for drilling 5, 15, 25, and 40 drill holes in equine cortical bone of MC/MT3 and P1 of 12 cadaver specimens. Drilling was carried out manually following surgical guidelines with continuous lavage. A routine full cleaning, washing and sterilisation cycle between every five drill holes was carried out. The roughness average (Ra) was determined over a measuring length of 1.5 mm. Light microscopy was carried out to identify signs of corrosion, and pores on the drill surface were counted and classified according to size. Variance analysis and correlations between the electropolished and untreated surfaces and roughness values, pores and drilling time and depth were calculated. Drill depth per drilling procedure (43mm-47mm) was not significantly different between respective groups. Electrochemical polishing increased roughness significantly by a mean of 0.2 μm in all subgroup comparisons. After electropolishing, the number of large porosities was also significantly increased, and the number of small porosities significantly decreased throughout the use of the drills. Electropolishing reduced corrosion signs in all stages of the study, with 36 unpolished drills and 3 electropolished drills showing corrosion. In conclusion: Electropolished bone drills potentially reduce the risk of infection due to reduced corrosion.

Keywords: equine cortical bone / fracture fixation / bone drill / stainless steel / electrochemical polishing

Die Oberfläche von chirurgischen Standardbohrern und elektropolierten Standardbohrern verändert sich mit der Verwendung im Cortex vom Röhrenknochen des Pferdes und nach routinemäßigen Reinigungs- und Sterilisationszyklen

Faktoren, die möglicherweise zur Entstehung von Implantat-assoziierten Infektionen beitragen, sind von immenser klinischer und wirtschaftlicher Bedeutung. Die vorliegende Studie hatte das Ziel, die Veränderungen an der Oberfläche von chirurgischen Bohrern zu untersuchen, die im Rahmen der typischen klinischen Verwendung im Pferdeknochen entstehen, wobei zwei Gruppen von Bohrern (Standardbohrer und elektropolierte Standardbohrer) verglichen wurden.Einhundert chirurgische Standardbohrer (Stahl Klassifikation AISI 440A) wurden für die Studie verwendet, wobei die Hälfte davon elektropoliert wurde (in HE 310, zwei Minuten lang). Die Bohrer wurden in der Folge in Gruppen verwendet um 5, 15, 25, und 40 Bohrlöcher in den langen Röhrenknochen von 12 randomisierten distalen Pferdeextremitäten (Röhrbein und Fesselbein) manuell zu bohren. Nach jeweils 5 Bohrlöchern wurde ein der klinischen Routine entsprechender Reinigungs- und Sterilisationszyklus durchgeführt. Als Maß für die Rauigkeit wurde über eine Länge von 1.5mm der roughness average (Ra) bestimmt, weiters wurden die Poren gezählt und in Größenklassen eingeteilt sowie Korrosionszeichen erhoben. Mittels Varianzanalyse wurden die Gruppen verglichen, und es wurden Korrelationen zwischen Rauhigkeit, Poren, Bohrzeiten und Bohrtiefen berechnet. Die Bohrtiefe pro 5 Bohrlöcher (43mm-47mm) war nicht signifkant unterschiedlich zwischen den Gruppen. Die Elektropolitur erhöhte die Rauhigkeit signifikant um im Durchschnitt 0.2 μm in allen Gruppen. Nach der Elektropolitur war die Anzahl großer Poren erhöht, und die Anzahl kleiner Poren reduziert, im Vergleich zu den unbehandelten Bohrern. Die Anzahl der kleinen Poren wurde mit der Bohrverwendung reduziert. Die Elektropolitur reduzierte die Korrosionszeichen in allen Bohrverwendungsgruppen, und 36 unpolierte aber nur 3 elektropolierte Bohrer zeigten Korrosionszeichen. Die Oberfläche von Bohrern in wiederholter Verwendung am Pferdeknochen stellt ein mögliches Risiko für die Entstehung von Infektionen dar, da sowohl Korrosion als auch oberflächliche Poren Gewebe schädigen können und auf der anderen Seite direkt zum Bakterienwachstum beitragen können. Die Elektropolitur von Bohrern, die zur wiederholten Verwendung am Pferd gedacht sind, führt zu einer starken Reduktion der Korrosion, und diese Reduktion der Korrosion bleibt auch bei starker Verwendung der Bohrer signifikant. Diese Korrosionsreduktion verringert das Risiko von post operativen Infektionen, weil keine Korrosionsprodukte im Knochen verbleiben und den Weg für bakterielle Infektionen durch Beeinträchtigung des Gewebes ebnen.

Schlüsselwörter: Pferdeknochen / Frakturbehandlung / Knochenbohrer / chirurgischer Stahl / elektrochemisches Polieren

Introduction

In orthopaedic surgery in human and veterinary medicine the cleanability and the corrosion resistance of the biomaterials used are essential. Although post-operative infection rates decreased within the last years, the overall number of infections increases due to the growing numbers of orthopaedic surgery making it necessary to improve the materials used further (*Ha* et al. 2005, *Subbiahdoss* et al. 2009).

Of all orthopaedic surgical materials, implants have been studied most frequently. However, other materials used during surgery such as drill bits or tapping bits may also contribute to the incidence of infection.

In implants there is ample evidence that material properties like surface finish and alloying elements also significantly influence bacterial attachment (*Gristina* et al. 1976, *Cordero* et al 1994, *Boulangé-Petermann* et al. 1997, *Gracia* et al 1997, *Medilanski* et al. 2002, *Ha* et al. 2005). No similar studies are available for materials, which are in short-term contact with bone, such as surgical drills. While implants are now also available in materials other than surgical grade steel, the instruments used for the implantation process are still made of medical stainless steel.

Bone drilling is one of the fundamental steps of many different complex procedures in orthopaedic surgery (*Bertollo* et al. 2008, *Bertollo* et al. 2010, *Vankipuram* et al. 2010). It is conducted with metallic drill bits in a suitable size to remove certain amounts of bone for implantation (*Bertollo* et al. 2008). The drilling procedure has been studied, and the main potential hazards identified with bone drilling for implantation were identified as: soft tissue injury caused by skiving, over-heating, metallic material release and pathogen propagation and metallic material transfer between patients (*Hobkirk* and *Rusiniak* 1978, *Eriksson* and *Albrektsson* 1983, *Eriksson* and *Albrektsson* 1984, *Iyer* et al. 1997a, *Iyer* et al. 1997b, *Hilbert* et al. 2003, *Ercoli* et al. 2004, *Bertollo* et al. 2008, *Yoshida* et al. 2009, *Bertollo* et al. 2010). Soft tissue injury and over-heating are problems that are related to the drill bit´s design. In contrast to the widely-used two-fluted drills, three-fluted drills have better properties concerning drilling of oblique bone holes because skiving occurs less frequently and is therefore a safer procedure in the vicinity of soft tissue (*Bertollo* et al. 2008). Over-heating of bone leads to bone necrosis and breakdown of the local microcirculation, the main technique to reduce this is continuous flushing of the drill bit and the drill hole with cool fluids. Even temperatures around 44-50°C for one minute reduce bone regeneration because of delayed periosteal membrane regeneration and therefore over-heating should be avoided (*Eriksson* and *Albrektsson* 1983, *Eriksson* and *Albrektsson* 1984, *Iyer* et al.1997a, *Iyer* et al. 1997b, *Ercoli* et al. 2004, *Yoshida* et al. 2009). Three-fluted drills show better cutting efficiency and a decreased drilling time that reduce heat production during drilling and support bone healing (*Bertollo* et al. 2010). The transfer of metallic material to the host is another problem coming up with bone drilling. There are two causes for metallic material transfer: Firstly, corrosion of the drill bits can decrease the material stability and lead to the release of metallic material during drilling. Corrosion resistance depends on the type of alloy composition, the manufacturing process and on the surface finish which can be modified by different procedures like grinding, bead blasting or electro-polishing which smoothen the surface and reduce bacterial attachment opportunities (*Hilbert* et al. 2003). Secondly, there can be transfer of metallic material during the drilling procedure as the drill material needs to resist to the mechanical resistance of the bone. This leads to the transfer of small amounts of metals into the bone surrounding the drill hole inducing electrochemical effects if there is a potential difference between the drill material and the implant material (*Hobkirk* and *Rusiniak* 1978).

Last but not least the transfer of pathogens and organic material positioned in cracks and crevices on the drill bit surface not reached by standard cleaning procedures represents a risk to a successful implantation (*Hilbert* et al. 2003). Micro-organisms can be transferred to another patient as drills are widely used repeatedly because of financial considerations (*Aasim* et al. 2006, Eterpi et al. 2009). This transfer depends on the efficiency of the cleaning and sterilization procedure in relation to the drill bit´s surface.

As the surface finish is an important factor determining corrosion resistance and rate of bacterial attachment many implants are electro-polished. Electrochemical polishing belongs to the contact-free electrochemical processes as micro-structural peaks of the surface are removed and so the surface is smoothened, and crevices and cracks in the surface are cleaned and opened for the subsequent use of cleaning agents. Also, electrochemically smoothened surfaces have a thicker and more uniform protective passive layer mostly consisting of chromium oxide compared to untreated surfaces. The corrosion resistance of such a thick passive layer increases the protection of the surface, and therefore smoothened passive stainless steel surfaces are less susceptible to corrosion attacks, resulting in an improved biocompatibility (*Beddoes* and *Bucci* 1999, *Arnold* and *Bailey* 2000, *Arnold* et al. 2004, *Gellér* et al. 2008).

The aim of this study is to investigate the influence of drilling of equine cortical bone on the surgical properties and surface qualities of standard surgical stainless steel drill bits which

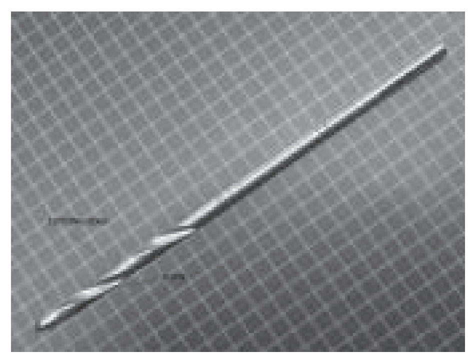

Fig. 1 Drill bits before electrochemical polishing showing flute and cutting edge

Fig. 2 Latero-medial radiograph of the same distal equine limb (bone 2) before drilling (A) and after drilling (B), the total drilling depth in this limb was 4672mm.

Table 1 Mean value (MV) and standard deviation (SD) in mm of drilling procedure bones

	metacarpal/ metatarsal bones	first phalanx bones
MV diameter (mm)	37.37	31.55
SD diameter (mm)	4.04	3.08
MV length in total (mm)	287.36	88.43
SD length in total (mm)	27.71	6.26
MV thickness of dorsal cortex (mm)	13.90	7.72
SD thickness of dorsal cortex (mm)	2.12	1.09

are in daily use in equine surgery and electropolished surgical stainless steel drill bits. The hypothesis investigated is that usage increases the number and size of surface defects in both types of surgical drill bits compared to unused drill bits.

Material and methods

Drills

One hundred two-fluted drills shown in Figure 1 were provided by company SYNTHES, Switzerland. The drills had a total length of 130 mm, the cutter part 50 mm and a shank diameter of 4.5 mm. According to the manufacturer´s information the drills were made of AISI 440A which equates to DIN 1.4109 and X70CrMo15. The manufacturing process included hardening and tempering to 52 +3/-0 HRC. The surface finish was performed by primary passivation ASTM A967, method Nitric 5, laser-labeling and secondary passivation.

Bones

Twelve metacarpal, metatarsal and first phalangeal bones of commercially slaughtered adult horses were randomly chosen for the drilling procedure. The bones were documented using x-ray before and after the drilling procedure as shown in Figure 2. Mean values (MV) and standard deviation (SD) of the anatomical specifications of the bones are shown in Table 1.

Microscopic Inspection (MI)

Three locations of the apex of each drill (Figure 3) were examined with a reflected-light microscope (BX51M with camera CAM XC30 and software Analysis Docu 5.1 from OLYMPUS, Tokio, Japan) at a 200-fold magnification. The examination area was 920.87 μm^2. Using the software, pores and scratches on the drill surface in the examination area were counted and classified into ten classes depending on their area size. The classes were divided as 0.01-5.00μm^2 (class -5μm^2), 5.01-10μm^2 (class -10μm^2), 10.01-20μm^2 (class -20μm^2), 20.01-30μm^2 (class -30μm^2), 30.01-40μm^2 (class -40μm^2), 40.01-50μm^2 (class -50μm^2), 50.01-100μm^2 (class -100μm^2), 100.01-600μm^2 (class -600μm^2), 600.01-800μm^2 (class -800μm^2) and 800.01-1000μm^2 (class -1000μm^2).

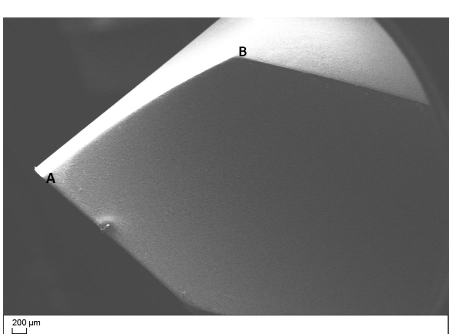

Fig. 3 Scanning Electron Microscopy picture of a two-fluted drill before drilling (Synthes, Switzerland), A: flute; B: cutting edge 1; (C: cutting edge 2 not seen on the picture).

Roughness Measurement (RM)

The roughness average (Ra) as the arithmetic mean of several absolute values over a certain measuring length (Henkel et al, 2007) was determined with a HOMMEL Tester T1000 (Hommel & Seitz, Viernheim, Germany) over a measuring length of 1.5 mm three times each on the flute and the cutting edge of each drill.

Scanning Electron Microscopy (SEM)

Drills were examined with Scanning Electron Microscopy (EVO 60XVP from Zeiss NTS Oberkochen, Germany, with software SmartSEM Version 5) to confirm the reflected-light microscopy results.

Electrochemical polishing (ECP)

This preparation step was carried out at the company HENKEL (Beiz- und Elektropoliertechnik GmbH & Co. KG, Waidhofen/Thaya, Austria). Overall 53 drills were electrochemically polished in HE 310 containing sulphuric, phosphoric and chromic acid for two minutes at 4.5 V and 3.7 A. This lead to an estimated surface abrasion of 4-6 μm according to the Faraday equivalent.

Drilling (DR)

Drills were randomly divided into 5 groups with 18 drills each, eliminating one remaining drill. Group 1 to 4 contained 18 drills each, 9 untreated (UT) and 9 electrochemically polished (EP). Group 5 consisting of 20 drills – 10 untreated and 10 electrochemically polished – which were forwarded for microbiological analysis (unpublished data). Before the drilling procedure started the 72 drills of group 1 to 4 were identified by manual engraving on the shaft and then sterilized. During the drilling procedure each group went through a different number of drilling cycles. One drilling cycle was defined as five drill holes plus washing and sterilization. The osseous drill holes were drilled with a standard motor drill (PSB 2000, „Bosch", Gerlingen, Germany). So the scheme was set as following: Group 1 (18 drills): one drilling cycle, group 2 (18 drills): three drilling cycles, group 3 (18 drills): five drilling cycles and group 4 (18 drills): eight drilling cycles. During the drilling the drills were manually cooled with cold tap water continuously flushed using sterile plastic syringes. Drill hole depth measuring was carried out with a routinely used depth gauge. Also, drill time was measured for each drill hole (AW-80-1AVES from „Casio", Tokio, Japan). For better differentiation the drills were named by the different experimental stages: UT (untreated) and pEP (prior to electrochemically polishing) in the UT (untreated) experimental step, EP (electrochemically polished) in the EP (electropolished) step and dUT and dEP in the DR (drilled) experimental stage.

The washing procedure was carried out in an automated washer (G7883CD from „Miele", Gütersloh, Germany) following EN ISO 15883-1 with the VarioTD washing programme using neodisher LaboClean FLA as washing agent and neodisher N as neutralizer (both from „Dr. Weigert", Hamburg, Germany). For sterilization each drill was packed separately (Medipeel from „Sengewald", Germany) and the sterilization took place in a „Getinge" steam autoclave with following parameters: 121°C, 1.2 bar in chamber and jacket, 2.3-2.4 bar steam supply for 55 minutes. Both the washing and the sterilization procedure followed the standard protocol of the surgical clinic.

Additional SEM / EDX- Analysis

Four drills (two out of group 5, one out of group 1 and one out of group 3) were inspected via SEM (QUANTA 200K from FEI, Hilsboro, OR) and energy-dispersive x-ray (EDX; PHOENIX from EDAX, with software Genesis 5.1, Mahwah, NJ) to carry out further material investigation concerning the alloy´s and manufacturing process qualities. SEM analyses were made for detailed information about the dissemination of impurities and inclusions which were then analyzed via EDX for their elemental composition.

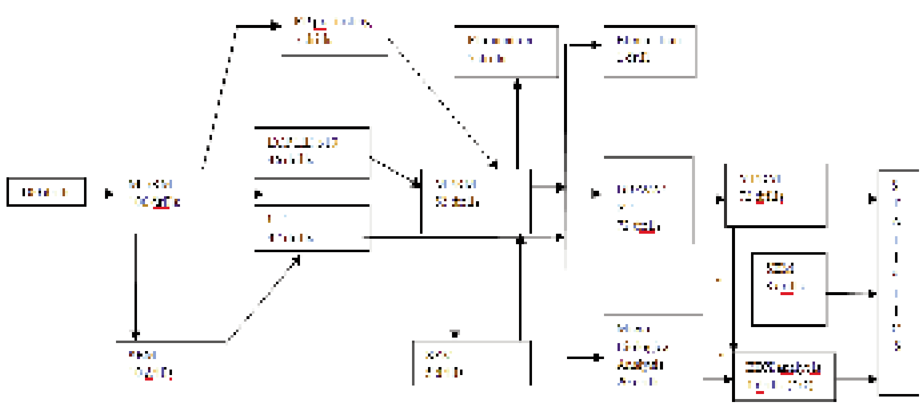

Fig. 4 Schematic overview of the experimental procedure of the drills. MI: microscopic inspection; RM: roughness measurement; ECP: electrochemical polishing, DR: drilling, WA: washing, ST: sterilization, SEM: scanning electron microscopy, EDX: energy dispersive x-ray, EP: electropolished, UT: untreated, HE310: brand name electrolyte

Table 2 Mean values (MV) and standard deviation (SD) of roughness average (Ra) on flutes and cutting edges (edge)

	group 1		group 2		group 3		group 4	
Untreated								
	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)
	MV (SD)	MV(SD)	MV (SD)	MV(SD)	MV (SD)	MV(SD)	MV (SD)	MV(SD)
Ra flute (µm)	0,16 (0,025)	0,16 (0,012)	0,14 (0,018)	0,15 (0,015)	0,15 (0,012)	0,16 (0,026)	0,17 (0,028)	0,15 (0,017)
Ra edge (µm)	0,19 (0,016)	0,19 (0,024)	0,18 (0,013)	0,19 (0,018)	0,19 (0,024)	0,20 (0,029)	0,18 (0,031)	0,19 (0,030)
Electrochemically polished								
	group 1		group 2		group 3		group 4	
	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)
	MV (SD)	MV(SD)	MV (SD)	MV(SD)	MV (SD)	MV(SD)	MV (SD)	MV(SD)
Ra flute (µm)		0,37 (0,039)^{EE}		0,38 (0,040)^{EE}		0,37 (0,052)^{EE}		0,38 (0,041)^{EE}
Ra edge (µm)		0,41 (0,088)^{EE}		0,42 (0,063)^{EE}		0,44 (0,027)^{EE}		0,42 (0,071)^{EE}
drilled								
	group 1		group 2		group 3		group 4	
	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)
	MV (SD)	MV(SD)	MV (SD)	MV(SD)	MV (SD)	MV(SD)	MV (SD)	MV(SD)
Ra flute (µm)	0,16 (0,033)**	0,37 (0,061)**	0,17 (0,032)**/^{D}	0,36 (0,057)**	0,15 (0,030)**	0,33 (0,046)**	0,16 (0,023)**	0,33 (0,056)**
Ra edge (µm)	0,19 (0,039)**	0,36 (0,043)**	0,18 (0,022)**	0,37 (0,082)**	0,21 (0,019)**/^{D}	0,36 (0,093)**/^{D}	0,19 (0,016)**	0.35 (0.058)**

* significantly different during same experimental stage ** highly significantly different during same experimental stage ^{D} significantly different after drilling, ^{DD} highly significantly different after drilling; ^{E} significantly different after electro polishing, ^{EE} highly significantly different after electro polishing

Data collection, data processing and statistical analysis

The different measuring procedures were repeated during the experimental set. A schematic overview is given in Figure 4. Data were analyzed using SPSS for Windows (version 17.0, SPSS Inc. Chicago, IL). First data were tested for Gaussian distribution. Variance analysis was performed and contingency tables were used to investigate the relationship between surface modification, roughness values, pores and drilling time and depth.

Results

Drilling time and depth

The drilling depth measurement revealed no significant differences between the in the UT and EP drilling sets used drills. The total drilling depth in group 1 was 214 mm ±8 (dUT) and 219 mm ±15 (dEP), in group 2 694 mm ± 9 (dUT) and 691±37 (dEP), in group 3 1182 mm ±36 (dUT) and 1184 ±54 (dEP) and in group 4 1838 mm ±36 (dUT) and 1850 mm ±67 (dEP). The total drilling times showed no significant differences in group 1 (dUT 41s±18; dEP 27s±10), in group 2 (dUT 104s±14; dEP 99s±11) and in group 3 (dUT 109s±19; dEP 101s±21). In group 4 drilling times of the dEP drills were significantly shorter (p=0,049; dUT 127s±11; dEP 116s±12). As shown in Figure 5 the total drilling depths were equal between UT and EP drills in the different groups.

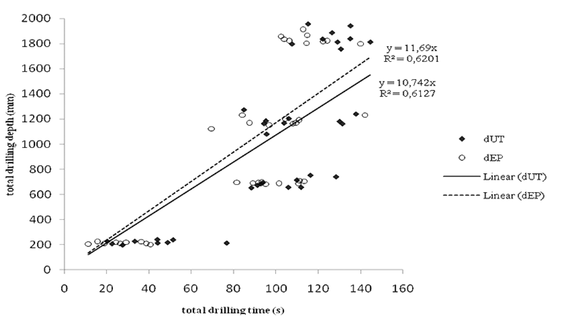

Fig 5 Correlation between total drilling time and total drilling depth; dUT: untreated drills, dEP: electrochemically polished drills

Roughness

Mean values and standard deviations of Ra on flutes and cutting edges of the drills during the different experimental stages are given in Table 2. The original state of the drill surfaces concerning roughness did not show any significant differences between UT and pEP drills neither on flutes nor cutting edges. Prior to drilling, the UT drills showed Ra ranging on the flutes between 0.12µm and 0.22µm and between 0.14µm and 0.25µm on the cutting edges. The ECP increased Ra significantly, which then ranged between 0.31µm and 0.46µm on flutes and 0.27µm and 0.55µm on cutting edges. The Ra measurement after drilling showed Ra for the dUT drills between 0.11µm and 0.22µm on flutes and 0.15µm and 0.29µm on cutting edges. The dEP drills showed a Ra range between 0.23µm and 0.46µm on flutes and 0.27µm and 0.53µm on cutting edges.

Porosities

Mean values and standard deviations of porosities in total on flutes and cutting edges of the drills during the different experimental stages are given in Table 3.The number of porosities in total was investigated microscopically and ranged in the UT experimental stage between 41 and 351 on flutes and 114 and 417 on cutting edges. ECP changed the number of porosities significantly and was between 68 and 258 on flutes and 104 and 256 on cutting edges. After dril-

Table 3 Mean values (MV) and standard deviations (SD) of porosities counted on flutes and cutting edges (edge);

	Untreated							
	group 1		group 2		group 3		group 4	
	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)
	MV (SD)	MV(SD)	MV (SD)	MV(SD)	MV (SD)	MV(SD)	MV (SD)	MV(SD)
flute	243 (69)	230 (69)	182 (63)*	248 (56)*	193 (61)	191 (93)	180 (78)	204 (80)
edge	236 (76)	240 (41)	195 (46)	237 (75)	220 (54)	204 (50)	226 (60)	298 (96)
	Electrochemically polished							
	group 1		group 2		group 3		group 4	
	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)
	MV (SD)	MV(SD)	MV (SD)	MV(SD)	MV (SD)	MV(SD)	MV (SD)	MV(SD)
flute		127 (37)[EE]		135 (50)[EE]		120 (49)[E]		136 (37)[E]
edge		170 (24)[EE]		190 (53)		182 (36)		176 (25)[EE]
	Drilled							
	group 1		group 2		group 3		group 4	
	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)	UT (n=9)	EP (n=9)
	MV (SD)	MV(SD)	MV (SD)	MV(SD)	MV (SD)	MV(SD)	MV (SD)	MV(SD)
flute	207 (39)*	156 (39)*	191 (39)	158 (36)	218 (36)*	181 (33)*/[D]	203 (41)*	158 (44)*
edge	214 (37)	199 (42)[D]	214 (46)	207 (30)	250 (21)**	193 (22)**	241 (35)	222 (25)[DD]

* significantly different during same experimental stage; ** highly significantly different during same experimental stage; [D] significantly different after drilling, [DD] highly significantly different after drilling; [E] significantly different after electro polishing, [EE] highly significantly different after electro polishing

Table 4 Mean numbers (MN) and standard deviation (SD) of porosities in the porosity-classes on flutes and cutting edges (edge);

				Untreated				
	group 1		group 2		group 3		group 4	
flute								
-5µm²	40 (14)	41 (15)	32 (9)	37 (9)	34 (11)	30 (13)	32 (13)	33 (12)
-10µm²	85 (23)	80 (29)	65 (23)**	91 (13)**	63 (17)	70 (33)	66 (28)	74 (27)
-20µm²	58 (17)	60 (12)	46 (18)	57 (17)	48 (17)	44 (26)	44 (20)	50 (24)
-30µm²	24 (9)	21 (6)	18 (8)	23 (8)	18 (7)	21 (13)	15 (7)	19 (9)
-40µm²	12 (4)	10 (4)	9 (6)	13 (5)	11 (5)	8 (4)	8 (6)	9 (5)
-50µm²	7 (3)	6 (4)	4 (2)**	8 (2)**	5 (3)	7 (4)	5 (4)	6 (3)
-100µm²	12 (9)	10 (5)	7 (4)	14 (10)	10 (6)	8 /5)	8 (5)	9 (6)
-600µm²	5 (5)	3 (3)	2 (2)	5 (5)	5 (3)	3 (2)	3 (4)	4 (3)
edge								
-5µm²	39 (13)	39 (9)	38 (8)	42 (14)	36 (10)	35 (7)	39 (11)	47 (15)
-10µm²	83 (26)	81 (11)	70 (14)	81 (25)	75 (18)	72 (16)	75 (19)	97 (34)
-20µm²	56 (18)	59 (10)	45 (15)	56 (18)	53 (13)	48 (14)	56 (14)	72 (24)
-30µm²	22 (9)	23 (5)	17 (6)*	24 (6)*	20 (6)	19 (6)	22 (10)*	32 (9)*
-40µm²	11 (5)	13 (4)	8 (3)	11 (4)	12 (4)	9 (4)	11 (4)	15 (5)
-50µm²	8 (3)	7 (3)	6 (2)	8 (3)	7 (3)	6 (2)	7 (3)	9 (3)
-100µm²	13 (5)	14 (4)	10 (4)	12 (6)	12 (2)	11 (6)	12 (4)*	19 (7)*
-600µm²	4 (2)	5 (2)	3 (1)	5 (5)	5 (3)	4 (3)	5 (4)	7 (4)
				Electrochemically polished				
	group 1		group 2		group 3		group 4	
flute								
-5µm²		14 (6)^{EE}		15 (10)^{EE}		13 (8)^{EE}		13 (6)^{EE}
-10µm²		29 (12)^{EE}		35 (17)^{EE}		30 (16)^{EE}		34 (12)^{EE}
-20µm²		27 (10)^{EE}		28 (14)^{E}		25 (14)^{E}		24 (6)^{EE}
-30µm²		13 (5)^{EE}		12 (4)^{E}		11 (5)^{E}		14 (3)
-40µm²		7 (3)		7 (3)^{E}		6 (3)		8 (4)
-50µm²		5 (2)		5 (3)		6 (3)		6 (2)
-100µm²		15 (4)		14 (6)		13 (3)^{E}		14 (6)
-600µm²		17 (5)^{EE}		18 (5)^{EE}		16 (5)^{EE}		22 (4)^{EE}
edge								
-5µm²		24 (5)^{EE}		25 (8)^{EE}		25 (5)^{E}		22 (5)^{EE}
-10µm²		46 (5)^{EE}		55 (21)^{E}		49 (11)^{EE}		48 (7)^{EE}
-20µm²		34 (7)^{EE}		42 (14)		35 (8)^{E}		37 (7)^{EE}
-30µm²		16 (3)^{EE}		17 (6)^{E}		16 (3)		16 (4)^{EE}
-40µm²		10 (3)		9 (4)		11 (3)		9 (1)^{EE}
-50µm²		7 (2)		7 (2)		7 (2)		6 (1)^{EE}
-100µm²		15 (3)		16 (4)		15 (3)		17 (5)
-600µm²		19 (4)^{EE}		18 (7)^{EE}		22 (7)^{EE}		21 (4)^{EE}
				Drilled				
	group 1		group 2		group 3		group 4	
flute								
-5µm²	37 (10)**	21 (10)**	34 (11)**	20 (9)**	43 (11)**	23 (12)**/^{D}	37 (6)**	20 (13)**
-10µm²	71 (17)**	41 (13)**	71 (16)**	46 (19)**	75 (12)**	50 (17)**/^{D}	77 (19)**	43 (19)**
-20µm²	48 (11)**	30 (10)**	46 (10)**	31 (9)**	50 (15)	40 (9)^{D}	48 (10)**	31 (14)**
-30µm²	18 (6)	14 (7)	16 (5)*	11 (3)*	17 (5)	15 (4)	17 (6)	15 (5)
-40µm²	8 (4)^{D}	8 (3)	8 (4)	6 (2)	10 (4)	9 (2)	7 (4)	8 (4)
-50µm²	7 (3)	7 (1)	5 (2)	6 (3)	7 (5)	6 (3)	4 (2)	6 (3)
-100µm²	13 (7)	15 (6)	8 (4)**	15 (5)**	11 (6)	16 (5)	9 (4)*	14 (3)*
-600µm²	5 (4)**	19 (3)**	4 (3)**	21 (5)**	5 (4)**	21 (7)**/^{D}	4 (4)**	21 (5)**
edge								
-5µm²	34 (7)	28 (11)	38 (8)**	29 (4)**	41 (10)**	25 (6)**	39 (6)**	29 (5)**/^{DD}
-10µm²	70 (17)	57 (16)^{D}	72 (13)*	59 (9)*	81 (12)**	52 (9)**	80 (9)*	66 (15)*/^{DD}
-20µm²	52 (10)*	42 (10)*/^{D}	51 (12)	44 (10)	59 (9)**	41 (8)**	56 (11)*	46 (8)*/^{DD}
-30µm²	22 (5)	19 (4)	18 (9)	20 (6)	25 (5)**	15 (3)**	22 (6)	20 (3)^{DD}
-40µm²	11 (4)	9 (2)	19 (4)	11 (4)	13 (3)*	9 (2)*	12 (5)	11 (2)^{DD}
-50µm²	6 (2)	7 (3)	8 (2)	7 (2)	9 (1)**	6 (1)**	8 (3)	7 (2)
-100µm²	12 (3)*	16 (3)*	12 (4)*	17 (4)*	16 (4)	17 (2)	16 (5)	18 (4)
-600µm²	6 (2)**	20 (6)**	5 (3)**	20 (3)**	6 (3)**	25 (4)**	6 (4)**	24 (6)**

ling, a minimum number of 112 and a maximum of 273 on flutes and 133 and 298 porosities on cutting edges on dUT drills and between 105 and 253 on flutes and between 135 and 273 porosities on cutting edges on dEP drills. Mean numbers and standard deviations of porosities in the different porosity-classes in total on flutes and cutting edges of the drills during the different experimental stages are given in Table 4.

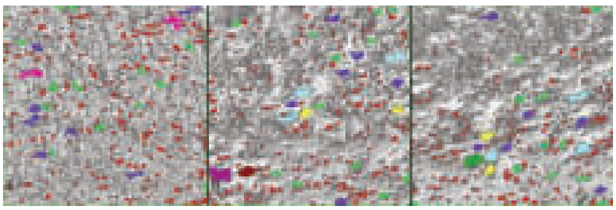

Fig 6 Reflected-light microscopy pictures of the surface of an pEP (untreated before electrochemical polishing and drilling, picture A), then EP (epolished before drilling, picture B) and dEP (epolished after drilling, picture C) drill (no. 18 of group 1) showing pores of different size on the investigation area of 920,87μm^2 coloured as classified into porosity-classes. Magnification 200. The pEP picture shows a total of 248 pores with an average size of 22.41μm^2, the EP picture shows a total number of 185 pores with an average size of 26.75μm^2 and the DR picture shows 231 pores in total with anaverage size of 26.01μm^2

In the UT experimental stage the largest number of pores was found in class -5μm^2 (5-60 pores on flutes, 19-70 pores on cutting edges), in class -10μm^2 (12-128 pores in flutes, 36-146 pores on cutting edges) and in class -20μm^2 (6-86 pores on flutes, 26-92 pores on cutting edges). The ECP had a deep impact on the different porosity classes on flutes and cutting edges. Especially small porosities with an area size between 0.01 and 20μm^2 were decreased, so on flutes class -5μm^2 showed 2-37 pores, class -10μm^2 13-74 pores and class -20μm^2 12-62 pores after ECP and on cutting edges class -5μm^2 revealed 14-38 pores, class -10μm^2 25-90 pores and class -20μm^2 18-63 pores. As an interesting fact, larger porosities were increased during ECP. After the drilling procedure the dEP drills and dUT drills still showed significant differences in the classes that had been affected by the ECP before. Furthermore, the drilling procedure increased the number of small pores. Especially class -10μm^2 was mostly affected and showed then 23-113 pores on flutes and 32-105 pores on cutting edges.

Classes -800μm^2 and -1000μm^2 of all experimental stages were eliminated from statistical analysis as only between 0 and 4 pores were counted, with a median of 0 in all drill groups.
A microscopic picture of a drill surface is given in Figure 6 (A: pEP, B: EP, C: dEP) and the changes of the numbers of pores in

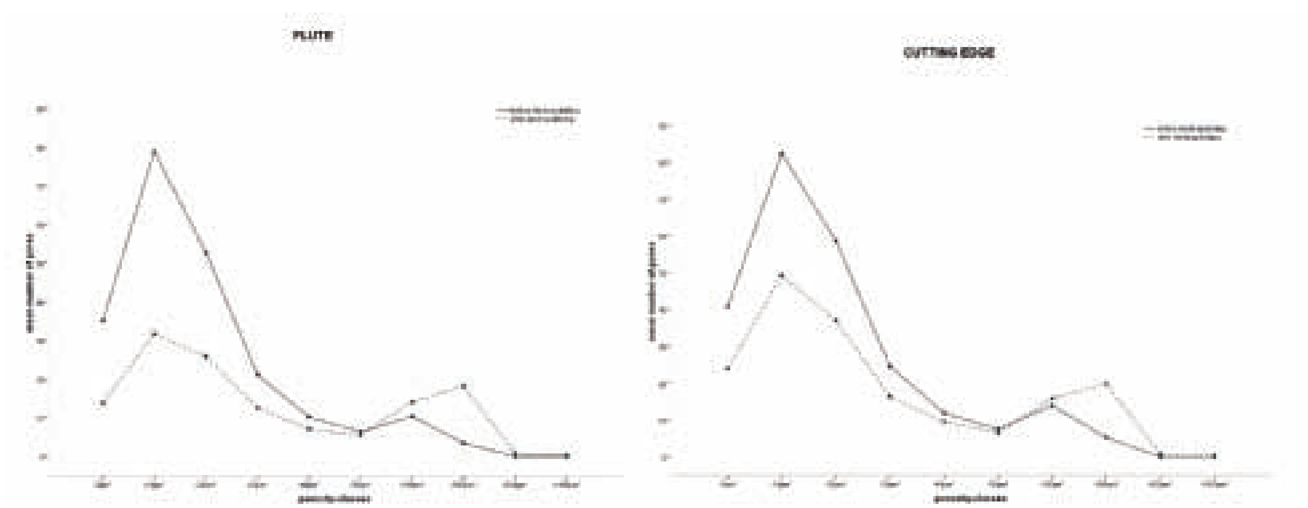

Fig. 7 Numbers of porosities in different porosity-classes on surgical stainless steels drills before and after electrochemical polishing.

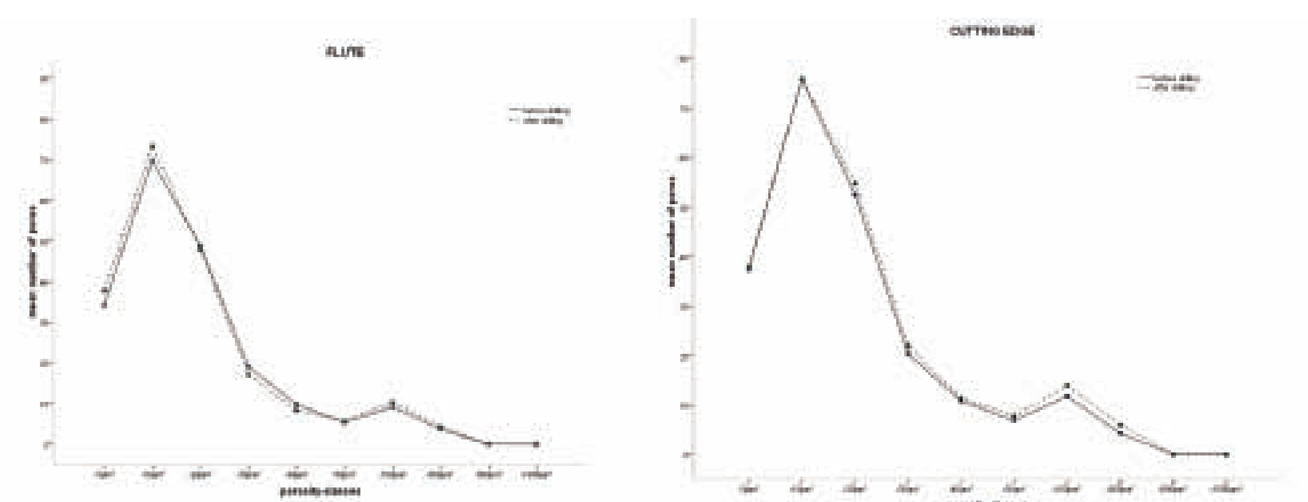

Fig. 8 Numbers of porosities in different porosity-classes on untreated surgical stainless steels drills before and after drilling

the different porosity-classes during electrochemical polishing is shown in Figure 7. The impact of drilling on the numbers of pores in the different porosity classes on flute and cutting edge of dUT and dEP drills is shown in Figure 8 and Figure 9.

EDX and SEM-Analysis

EDX and SEM analysis made of two different drills UT/EP revealed a large number of inclusions within the alloy as shown in Figure 10 A/B and Figure 11.

Corrosion signs

The microscopic investigation of corrosion signs showed highly significant differences ($p<0.001$) between UT and EP in all groups, with all 36 investigated dUT drills and 3 dEP drills of the groups 1, 2, 3 and 4 showing brownish spots on the surface. SEM and EDX analysis of one drill revealed oxygen in the brownish spots on the drill surface, confirming presence of corrosion products as shown in Figure 12.

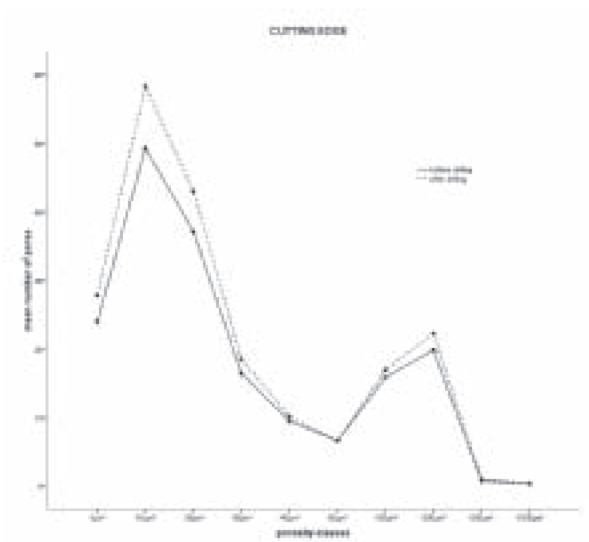

Fig. 9 Numbers of porosities in different porosity-classes on electrochemically polished surgical stainless steels drills before and after drilling

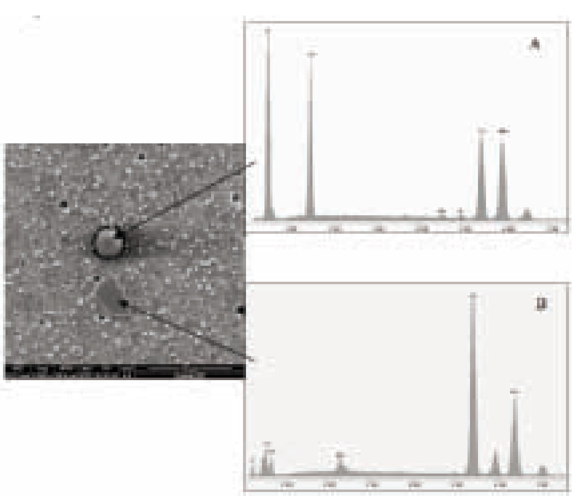

Fig. 10 Cross section SEM picture magnification 5000, and EDX analysis of an UT (Untreated) drill. Picture A is an EDX spectrum of the round inclusion shown in the SEM picture revealing aluminium oxide from the manufacturing process; picture B is an EDX spectrum of the large inclusion shown in the SEM picture revealing a high amount of chrome which is a representative sign for chromium oxide in such alloy.

Discussion

In our study commercially available, 2-fluted drill bits were investigated as they are commonly used in equine orthopaedic surgery. Three-fluted drill bits have been studied concerning their drilling performance and they are superior in several aspects, however, two-fluted drills are still commonly used drilling instruments (*Bertollo* et al. 2008, *Bertollo* et al. 2010), also in equine surgery. Focussing on materials in every day clinical routine makes the results more relevant to the surgical field. As a surface modifying technique, electrochemical polishing was chosen as it is used to smoothen and improve the alloy surface, of metal surfaces used for surgical applications, semi-conductors and within the food processing industry (*Beddoes* and *Bucci* 1999, *Stoodley* et al. 2005, *Gellér* et al. 2008, *Arnold* and *Bailey* 2000, *Arnold* et al. 2004, *Lin* and *Hu* 2008, *Hocheng* et al. 2001, *Chow* et al. 2008). In a follow up study to the present one, the influence of electrochemical polishing and its impact on the bacterial attachment will be studied.

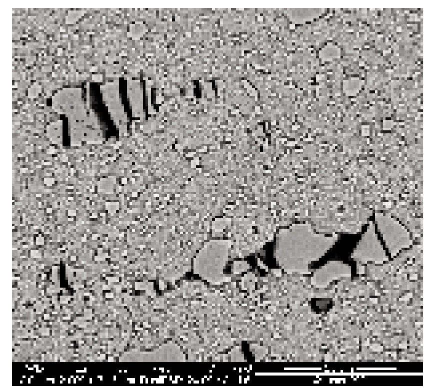

Fig. 11 Longitudinal section: SEM picture of an UT (Untreated) drill, magnification 2000. The picture shows a large number of inclusions of different sizes in the alloy.

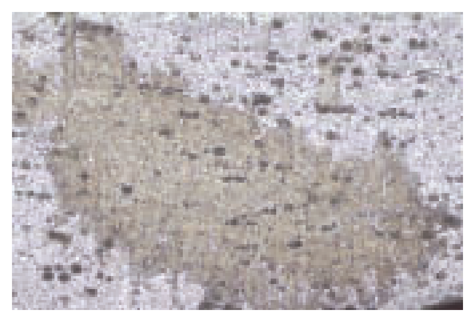

Fig. 12 Reflected-light microscopic picture of a brownish spot on a dUT drill surface, magnification 200, indicating corrosion.

To compare the roughness and the porosities between new and used drills we chose a manual drilling technique on metacarpal and metatarsal bones of adult horses. The selected maximum of 40 drilling holes and a maximum drilling depth of 1958 mm with 9 cleaning and sterilization cycles can be expected to be routine usage for a single drill bit. As equine bone density and cortex thickness outmatch bones from humans and from smaller mammal species, the changes documented on the used drill bits are possibly larger than changes caused by similar drill depths and drilling procedure numbers in human surgery. The EP drills allowed significantly faster drilling in group 8, possibly indicating that EP drills resist bone stability and drilling demands better and longer than UT drills. However, only one professional orthopaedic surgeon carried out all the drilling, and therefore these results would ideally be confirmed using an automated and standardized drilling apparatus in a larger scale study, to evaluate its relevance.

Roughness is an important characteristic for the stainless steel surface topography, and for the identification of roughness the two-dimensional roughness average (Ra) is described as a well established and cost effective method. Compared to modern three-dimensional methods as e.g. Atomic Force Microscopy (AFM) it is not as suitable for investigation of surface topographies in detail, but today Ra is still the most frequently used roughness parameter in material industry allowing easy comparison of the data obtained in our study (*Ungersbock* and *Rahn* 1994, *Stout* and *Blunt* 1995, *Arnold* and *Bailey* 2000, *Arnold* et al. 2004). Measurements were completed by microscopic porosity counts. Porosities in the stainless steel surface influence the roughness as they represent slots and crevices which can be seen as irregularities and roughness measurement influencing characteristic of the surface. The relationship between the classes of porosities and the Ra shows that due to electrochemical polishing roughness is significantly increased whereas the porosities of an area size between 5 and 600μm^2 are decreased. Larger porosities are increased. Roughness and porosities depend on the material used for the drills, with the drill alloy (AISI 440A) containing between 0.65-0.75% carbon and 13-15% chromium. Both the carbon and the chromium have important functions-the carbon is set to the alloy to improve the hardness and cutting properties of the drill and the chromium is the main element for building up the protective passive layer consisting of chromium oxide (*Fajers* et al. 1968). Stainless steel alloys must contain more than 11% chromium in the alloy to provide a protective passive layer for increased corrosion resistance. As chromium is a very reactive element it binds to the carbon in our specific stainless steel alloy to form highly resistant chromium carbides. 0.1% of carbon in the alloy is able to bind 2% of chromium (*Henkel* et al. 2007). During the ECP the chromium carbides react in a different way than their surrounding areas. Chromium carbides act as one large element better resisting the abrasion during the electrochemical polishing. They are surrounded by other alloying elements which are removed during electrochemical polishing. So the chromium carbides are eroded and then subsequently rinsed out. In our study this is documented by the reduction of smaller porosities, representing small crevices or small carbides, after the electrochemical polishing procedure. Larger porosities are increased after the electrochemical polishing as they result from the rinsing out of large chromium carbides. SEM and EDX analysis confirm this. Consequently, the use of a fine-grained stainless steel alloy for surgical applications may be more suitable in order to reduce the porosities.

Corrosion resistance is an important quality especially for reusable surgical instruments (*Shah* and *Bernardo* 2002). In the present study 36 dUT and 36 dEP drills were inspected microscopically for corrosion signs. The results were highly significant, namely independent from their group all 36 dUT drills showed a multitude of brownish spots on their surface whereas only 3 dEP out of 36 did. This shows that electrochemical polishing was successfully increasing the corrosion resistance of stainless steel as described in other studies (*Hocheng* et al. 2001, *Arnold* et al. 2004, *Shahryari* et al. 2008). Corrosion signs were determined microscopically, and the visual identification of corrosion as characteristic brownish spots on the stainless steel surface has been successfully used in many studies. (*Sandrik* und *Wragg* 1970, *Shah* und *Bernardo* 2002, *O'Hoy* et al. 2003, *Arnold* et al. 2004; *Wichelhaus* et al. 2004, *Henkel* et al. 2007). Microscopic pictures of each drill were made and classified concerning the existence of such spots. The presence of oxygen in the spots either as part of an oxide or a hydroxide molecule, as determined in the second step EDX analysis of two drills, proved that the brownish spots were corrosion products. The absence of corrosion signs in the new and the appearance of corrosion signs in the used drill bits show that high pressures and temperatures in the autoclave do have a corroding influence on the stainless steel surface. This may even be more relevant, if the time that the surgical instruments are unused between procedures is larger than in the present study, where drilling and sterilization procedures were carried out within five days and the microscopic inspection took place within the following three days. Consequently, the corrosion documented in this study probably leads to an underestimation of corrosion in real life situations, where drills are not necessarily used four to five times a day but stored for days or weeks between surgeries. The reason for the decreased corrosion resistance of the dUT drills may also be the formation of chromium carbides in the alloy, as in the areas surrounding chromium carbides chromium depletion occurs and thus chromium as the most important element for the protective passive layer is no longer available (*Henkel* et al. 2007). Studies investigating stainless steel surfaces concerning their corrosion resistance during autoclave sterilization found corrosion signs after autoclave sterilization on different types of stainless and carbon steels but simultaneously observed the corrosion resistance increasing properties of different inhibitors and amines (*Fajers* et al. 1968, *Sandrik* und *Wragg* 1970, *Wichelhaus* et al. 2004).

To investigate the clinical effect of an increased corrosion resistance further studies are planned, as the accumulation of corrosion products in the bone may have deleterious effects on bone healing and susceptibility for bone infection.

In the present study the original drill material is the most important parameter determining the drill quality. Electrochemical polishing was a significant influence on the resulting drill quality. Surprisingly, the roughness and porosities of the flutes and the cutting edges of both the UT and the EP drills remained similar independent from their drill usage, being apparently usage independent. Corrosion as a major determinant of quality was significantly reduced after the electrochemical polishing process, and therefore a high quality surface finish like electrochemical polishing should become standard in human and veterinary surgery in order to reduce drilling associated complications.

Conclusions

The surface of these routinely used surgical drills show the potential to be a hazard in surgical drilling of equine bone, as both the corrosion and the surface porosities have the potential to detrimentally affect the living bone tissue and to have a beneficiary effect on microbial growth and attachment to the implants. Electropolishing of drills intended for repeated use may be advantageous as it leads to a marked reduction of corrosion signs that is maintained throughout heavy use of the drill, including washing and sterilization procedures. The change in the pore sizes cannot be directly related to surgical benefits, and further studies are required to determine whether larger pore sizes improve cleanability or lead to more biological material retained during use.

References

Aasim S. A., Mellor A. C. and *Qualtrough A. J. E.* (2006) The effect of pre-soaking and time in the ultrasonic cleaner on the cleanliness of sterilized endodontic files. Int. Endod. J. 39, 143-149

Arnold J. W. and *Bailey G. W.* (2000) Surface finishes on stainless steel reduce bacterial attachment and early biofilm formation: Scanning electron and atomic force microscopy study. Poult. Sci. 79, 1839-1845

Arnold J. W., Boothe D. H., Suzuki O. and *Bailey G. W.* (2004) Multiple imaging techniques demonstrate the manipulation of surfaces to reduce bacterial contamination and corrosion. Microscopy-Oxford 216, 215-221

Beddoes J. and *Bucci K.* (1999) The influence of surface condition on the localized corrosion of 316L stainless steel orthopaedic implants. J. Mater Sci. Mater. Med. 10, 389-394

Bertollo N., Gothelf T. K. and *Walsh W. R.* (2008) 3-Fluted orthopaedic drills exhibit superior bending stiffness to their 2-fluted rivals: Clinical implications for targeting ability and the incidence of drill-bit failure. Injury 39, 734-741

Bertollo N., Milne H. R. M., Ellis L. P., Stephens P. C., Gillies R. M. and *Walsh W. R.* (2010) A comparison of the thermal properties of 2- and 3-fluted drills and the effects on bone cell viability and screw pull-out strength in an ovine model. Clin. Biomech. 25, 613-617

Boulangé-Petermann L., Rault J. and *Bellon-Fontaine M.* (1997) Adhesion of Streptococcus thermophilus to stainless steel with different surface topography and roughness. Biofouling 11, 201-216

Chow H. M., Lee S. M. and *Yang L. D.* (2008) Machining characteristic study of friction drilling on AISI 304 stainless steel. J. Mater. Process. Technol. 207, 180-186.

Cordero J., Munuera L. and *Folgueira M. D.* (1994) Influence of metal implants on infection: An experimental study in rabbits. J. Bone Joint. Surg. Surgery - Series B 76, 717-720

Ercoli C., Funkenbusch P. D., Lee H., Moss M. E. and *Graser G. N.* (2004) The influence of drill wear on cutting efficiency and heat production during osteotomy preparation for dental implants: A study of drill durability. Int. J. Oral Maxillofac. Implants 19, 335-349

Eriksson A. R. and *Albrektsson T.* (1983) Temperature threshold levels for heat-induced bone tissue injury: A vital-microscopic study in the rabbit. J. Prosthet. Dent. 50, 101-107

Eriksson R. A. and *Albrektsson T.* (1984) The effect of heat on bone regeneration: An experimental study in the rabbit using the bone growth chamber. J. Oral and Maxillofac. Surg. 42, 705-711

Eterpi M., McDonnell G. and *Thomas V.* (2009) Disinfection efficacy against parvoviruses compared with reference viruses. J. Hosp. Infect. 73, 64-70.

Fajers C. M., Holmlund L. G. and *Stenman E.* (1968) Corrosion during autoclave sterilization. II. Volatile organic amines as corrosion inhibitors. Acta. Odontol. Scand. 26, 23-34

Gellér Z. E., Albrecht K. and *Dobránszky J.* (2008) Electropolishing of coronary stents. Mat. Sci. Forum 589, 367-372

Gracia E., Fernández A., Conchello P., Laclériga A., Paniagua L., Seral F. and *Amorena B.* (1997) Adherence of Staphylococcus aureus slime-producing strain variants to biomaterials used in orthopaedic surgery. Int. Orthop. 21, 46-51

Gristina A. G., Rovere G. D., Shoji H. and *Nicastro J.* (1976) An in vitro study of bacterial response to inert and reactive metals and to methyl methacrylate. J. Biomed. Mater. Res. 10, 273-281

Ha K. Y., Chung Y. G. and *Ryoo S. J.* (2005) Adherence and biofilm formation of Staphylococcus epidermidis and Mycobacterium tuberculosis on various spinal implants. Spine 30, 38-43

Henkel G., Stienecker F. and *Wesch M.* (2007) Lexikon der Pharmatechnologie: Werkstoffe und Verfahren. 1st ed. Aulendorf: Editio Cantor Verlag

Hilbert L. R., Bagge-Ravn D., Kold J. and *Gram L.* (2003) Influence of surface roughness of stainless steel on microbial adhesion and corrosion resistance. Int. Biodeterior. Biodegrad. 52, 175-185

Hobkirk J. A. and *Rusiniak K.* (1978) Metallic contamination of bone during drilling procedures. J. Oral. Surg. 36, 356-360

Hocheng H., Kao P. S. and *Chen Y. F.* (2001) Electropolishing of 316L stainless steel for anticorrosion passivation. JMEPEG 10, 414-418

Iyer S., Weiss C. and *Mehta A.* (1997) Effects of Drill Speed on Heat Production and the Rate and Quality of Bone Formation in Dental Implant Osteotomies. Part I: Relationship between Drill Speed and Heat Production. Int. J. Prosthodont. 10, 411-414

Iyer S., Weiss C. and *Mehta A.* (1997) Effects of Drill Speed on Heat Production and the Rate and Quality of Bone Formation in Dental Implant Osteotomies. Part II: Relationship between Drill Speed and Healing. Int. J. Prosthodont. 10, 536-540

Lin C. C. and *Hu C. C.* (2008) Electropolishing of 304 stainless steel: Surface roughness control using experimental design strategies and a summarized electropolishing model. Electrochim. Acta. 53, 3356-3363

Medilanski E., Kaufmann K., Wick L. Y., Wanner O. and *Harms H.* (2002) Influence of the surface topography of stainless steel on bacterial adhesion. Biofouling 18, 193-203

O'Hoy P. Y. Z., Messer H. H. and *Palamara J. E. A.* (2003) The effect of cleaning procedures on fracture properties and corrosion of NiTi files. Int. Endod. J. 36, 724-732

Sandrik J L. and *Wragg L. E.* (1970) Corrosion effects of three sterilization methods on experimental implant surfaces. J. Biomed. Mater. Res. 4, 275-277

Shah S. and *Bernardo M.* (2002) Corrosion protection of reusable surgical instruments. Biomedical Instrumentation and Technology 36, 318-324

Shahryari A., Omanovic S. and *Szpunar J. A.* (2008) Electrochemical formation of highly pitting resistant passive films on a biomedical grade 316LVM stainless steel surface. Materials Science & Engineering C-Biomimetic and Supramolecular Systems 28, 94-106

Stoodley P., Kathju S., Hu F. Z., Erdos G., Levenson J. E., Mehta N., Dice B., Johnson S., Hall-Stoodley L., Nistico L., Sotereanos N., Sewecke J., Post J. C. and *Ehrlich G. A.* (2005) Molecular and Imaging techniques for bacterial biofilms in joint arthroplasty infections. Clin. Orthop. 31-40

Stout K. J. and *Blunt L.* (1995) Nanometers to Micrometers – 3-Dimensional Surface Measurement in Bioengineering. Surf. Coat. Technol. 71, 69-81

Subbiahdoss G., Kuijer R., Grijpma D. W., van der Mei H. C. and *Busscher H. J.* (2009) Microbial biofilm growth vs. tissue integration: „The race for the surface" experimentally studied. Acta Biomaterialia 5, 1399-1404

Ungersbock A. and *Rahn B.* (1994) Methods to characterize the surface roughness of metallic implants. J. Mater. Sci. Mater. Med. 5, 434-440

Vankipuram M., Kahol K., McLaren A. and *Panchanathan S.* (2010) A virtual reality simulator for orthopedic basic skills: A design and validation study. J. Biomed. Inform. 43, 661-668

Wichelhaus A., Brauchle G., Mertmann M. and *Sander F. G.* (2004) Corrosion of orthodontic pliers using different sterilization procedures. J. Orofac. Orthop. 65, 501-511

Yoshida K., Uoshima K., Oda K. and *Maeda T.* (2009) Influence of heat stress to matrix on bone formation. Clin. Oral. Implants. Res. 20, 782-790

Ao. Univ.-Prof. Dr.med.vet. Theresia Licka
Klinik für Pferde, Department für Kleintiere und Pferde
Veterinärmedizinische Universität
Veterinärplatz 1, 1210 Wien, Austria
theresia.licka@vetmeduni.ac.at