Int J Legal Med (2011) 125:21–26
DOI 10.1007/s00414-009-0394-z

ORIGINAL ARTICLE

Release of metals from osteosynthesis implants as a method for identification: post-autopsy histopathological and ultrastructural forensic study

Elisa Palazzo · Salvatore Andreola · Alessio Battistini · Guendalina Gentile · Riccardo Zoja

Received: 13 October 2009 / Accepted: 9 November 2009 / Published online: 3 December 2009
© Springer-Verlag 2009

Abstract Metal structures—especially of stainless steel, titanium and their alloys (biomaterials)—are widely used in orthopaedic practice and the subject of constant study in bioengineering and preventive medicine. This study presents the first experience of forensic research into the presence of permanent tissue variations around metal implants in various bone structures for the purpose of identification, with particular reference to skeletal remains or severely decomposed corpses in the absence of other identifying elements. The evaluation was conducted on 12 corpses who had undergone osteosynthesis intra-vitam, whose implants were still in place or had been removed, in comparison with five controls who had never undergone osteosynthesis. Bone fragments taken during autopsy were subjected to histopathological and scanning electron microscope–energy dispersive electroscopy examination in order to reveal and characterise any metal particles originating from osteosynthesis. The study enabled the discovery of intra-bone metal particles in tissues treated by osteosynthesis even in bone areas where the implants had been removed and even where there were no longer any radiological signs of their application. These results are therefore of considerable forensic importance, especially in the area of identification, providing a valid means of recognition beyond that of the well-established use of in situ metal implants.

Keywords Forensic medicine · Osteosynthesis implants · Metal residues · Bone · Identification

E. Palazzo · S. Andreola · A. Battistini · G. Gentile · R. Zoja (✉)
Dipartimento di Morfologia Umana e Scienze Biomediche,
Sezione di Medicina Legale e delle Assicurazioni,
Università degli Studi di Milano,
Via Luigi Mangiagalli, 37,
20133 Milan, Italy
e-mail: riccardo.zoia@unimi.it

Introduction

Metal osteosynthesis implants, made from metal, especially stainless steel, titanium and related alloys, i.e. biomaterials designed to be included in a biological system and to support or replace an anatomic function [1, 2], are used in the treatment of bone fractures; their application can provide a temporary surgical fixation of bones, removed once the fracture has repaired, or a permanent, irremovable fixation. The study of interaction between these structures and human tissues had the aim to establish any harmful long-term metabolic effects, mostly on animal samples [3–9] and, on humans, in the areas of maxillofacial and orthodontic surgery [10–13]. In forensic work, studies generally focus on autopsy cases where the presence of metal implants still in situ can provide important information, especially with respect to identification [14]. There have in fact been numerous reports in which the victims of mass disasters, among others, have been identified through techniques such as radiography or computed tomography or analysis of dental amalgams and resins by scanning electron microscope (SEM)–energy dispersive electroscopy [15–19]. This study aimed to establish if bone tissues undergo any permanent modifications following the application and subsequent removal of such implants, whose technical documentation might provide robust evidence for personal identification in relation to case history, even in the absence of detectable signs of the previous bone disorder or fixation surgery.

Materials and methods

Between October 2007 and February 2009, careful pre-autopsy analysis of case histories and documentary evidence was used to identify, from all cases undergoing autopsy at the Istituto di Medicina Legale at the University of Milan, corpses with osteosynthesis implants still in situ at the time of death or which had been fitted with such implants that had then been removed during the victim's lifetime. Cases treated with external fixation devices (such as Ilizarov fixators) were excluded in order to avoid any doubt over the exact correspondence between the bone lesion and the application of the osteosynthesis implant. Twelve cases meeting the study's requisites were selected: six male and six female, aged 30 to 79 years, all treated for fractures and from 2 of which the implant had already been removed: in case 3, the metal implants had been fixed for 23 months, and in case 10 for 19 months. The medical records of the osteosynthesis procedure carried out in orthopaedic hospital facilities were available for examination in eight cases. Table 1 reports the data for the cases in consideration. The selected cases were compared against five controls, consisting of autopsies of cases of natural death in people aged between 32 and 63 years who had never undergone orthopaedic surgery and were not suffering from any skeletal disorders (two cases of natural cerebral haemorrhage, one of cardiac rupture during acute myocardial infarction, one of coronary thrombosis and one of acute cardiogenic pulmonary oedema). Before autopsy, corpses underwent radiographic examination to locate in situ implants and examine the area of surgery. Case 10 was the only case in which radiographic examination showed no signs of the previous application of metal screws or noticeable post-fracture alterations to the malleolar morphology but only slight signs of osteoarthritis of the talocalcaneal articulation. The autopsy was then carried out with bone samples removed from the fixation areas using the following procedure: A 2-cm bone fragment was taken from the fixation area (whether the implant was present or had been removed) and a second sample of the same size was taken at a distance of 2.5 cm from the first; where the malleolus was involved, the entire bone was removed. The samples were taken using a KCC204 oscillating saw (Likn Superabrasive Co. Ltd.) with a ceramic blade, used in preference to a metal blade to avoid sample contamination. Similar bone samples were taken from the humeral, ulnar, radial, femoral and tibial shafts of the control cases: samples were taken using a metal blade from two of these samples to evaluate any metal artefacts produced by the saw blade itself. Each sample was divided into two: one for optical microscope examination and the other for Scanning electron microscope–energy dispersion spectrometry (SEM/EDS) examination for quantitative analysis. Half of the optical microscope samples were decalcified with formic acid (80 ml concentrated hydrochloric acid, 100 ml formic acid 90%, brought to volume (1,000 ml) with double-distilled water) and the other half with nitric acid 10% (10 ml concentrated nitric acid brought to volume (100 ml) with double-distilled water). All samples were then fixed in buffered formaldehyde 10%, washed under running water, dehydrated, embedded in paraffin, cut into 5-μm sections by microtome and stained with haematoxylin–eosin. They were then examined under transmitted and polarised light under the optical microscope Leica DMR, with digital images being taken of the relevant areas with Leica DC 300F digital camera. Samples for SEM analysis were fixed in buffered formalin 10%, dehydrated in absolute ethanol and then coated with a thin layer of gold (a few Ångstrom or tenths of a micrometre) with an Edwards Scancoat Six to maximise secondary electron yield. Back-scattered electron imaging and analysis required a carbon source in order to coat

Table 1 Overview of the cases under investigation in relation to the characteristics of the osteosynthesis

Case°	Sex	Age	Osteosynthesis implants	Place	Ostheosynthesis in situ: months of permanence	Removed osteosynthesis: months remotion/autopsy
1	M	64	Medullar rod	Femur	23	
2	M	58	Plate and six screws	Humeral shaft	61	
3	F	50	Plate and five screws	Tibial plate		52
4	F	30	Plate and four screws	Ulna	15	
5	M	49	Plate and six screws	Humeral shaft	20	
6	F	60	Medullar rod	Femur	15	
7	F	53	Plate and eight screws	Femoral shaft	39	
8	M	77	Three screws	Tibial malleolus	11	
9	M	78	Medullar rod	Femur	13	
10	M	46	One screw	Tibial malleolus		76
11	F	79	Total hip replacement	Hip	23	
12	F	62	Plate and seven screws	Femoral shaft	46	

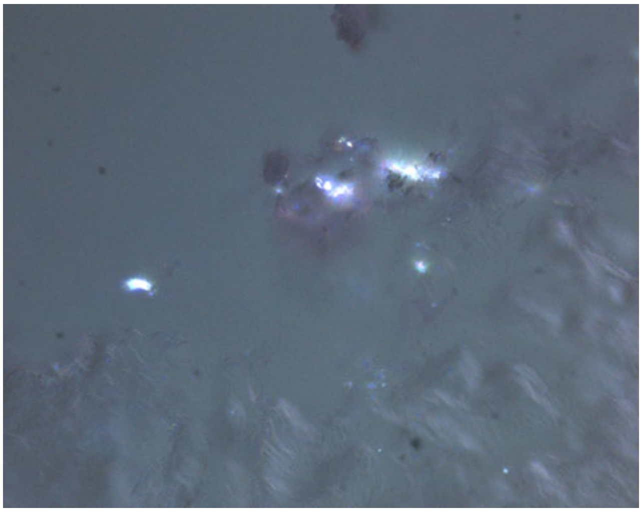


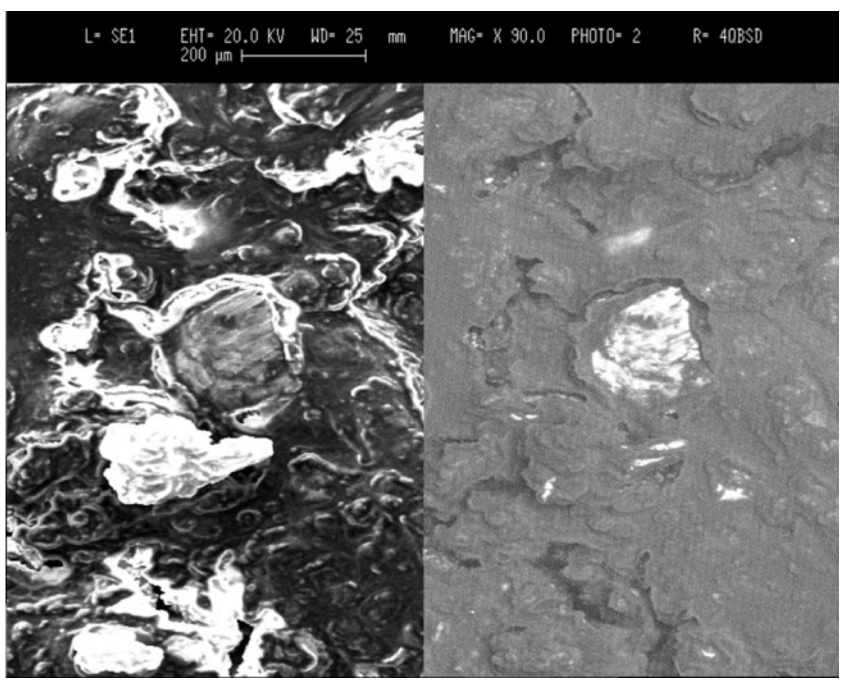
Fig. 1 Polarised light examination ×1,000: clear birefringence of both isolated granules and aggregates, with slight local degeneration of the bone structures along one edge of the sample

samples with a thin layer to induce their metallisation. Examinations were carried out using: Cambridge Stereoscan 360 with electron gun, vacuum pump and image acquisition software; EDS [energy dispersive (X-ray) spectrometry] with detector resolution from 138 eV to 5.9 keV, Oxford Link Pentafet to identify any metallic components in the bone fragments and an Oxford Link Isis console for display of the composition spectra.

Results

Optical microscope examination of the samples taken from the fixation site provided positive identification of four cases under transmitted light and eight cases under polarised light. The two cases where the osteosynthesis had been previously removed and another two cases (ulna and tibial malleolus) were found negative. Under transmitted light, the biomaterial particles appeared as small, dispersed, isolated granules or larger aggregates of amorphous blackish material. Under polarised light, there was a clear birefringence with both the scattered particles and aggregated material located in the bone structure: occasionally, tiny fragments with a clearly birefringent appearance were also seen, under both transmitted and polarised light, in the fibroadipose tissue surrounding the bone, incorporated in the connective tissues (Fig. 1). These findings are similar to those reported in the literature for studies of maxillofacial and orthodontic implants and prostheses [5, 7, 20, 21]. No granulomatous inflammation caused by the foreign body was seen in any case [13, 20, 21]. Optical microscope examination of samples taken further from the fixation area was negative in all cases. All control cases were negative for foreign bodies. SEM analysis was positive for nine of the 12 samples taken from the fixation site and in four samples (hip replacement, humeral shaft and two femoral rods) taken at a distance; in both cases in which the fixation device had already been removed (tibial plate, tibial malleolus), the bone tissue tested positive for

Fig. 2 Back-scattered electron image of metal residues incorporated in the bone trabeculae



metal residues. In the positive cases, fragments of various shapes and sizes were found, incorporated in the depths of the bone trabeculae (Fig. 2). EDS analysis revealed the composition of the osteosynthesis implant components (Fig. 3) identified as iron (70%), titanium (20%), nickel, cobalt and chrome (10%). SEM examination of the five control cases proved negative for the samples taken using a circular saw fitted with a ceramic blade, while iron was found in those taken with a metal blade: clear signs of grooving were seen, attributable to the action of the oscillation saw on the cut bone surface, and, most importantly, metal was found on the outer bone surface only, in contrast with the positive cases where metal particles were found deep down and incorporated into the structure of the bone trabeculae, thus demonstrating their intra-vitam origin (Table 2).

Discussion

The release of metal from osteosynthesis implants used in treatment (especially of traumatic fractures) has been studied in animals [3, 8, 9], in clinical dentistry and in maxillofacial surgery [2, 4–6, 8, 10–13, 20]. In the latter, various studies have also looked into its possible application in forensic odontology [18, 19], suggesting not only the well-established role in the identification of corpses when metal implants are found in situ but also innovative methods based on bioengineering techniques [22], computerised virtual facial reconstructions [23] and 2D–3D superimposition techniques [24]. This study is the first forensic research into whether metal implants cause any permanent tissue variations to bone structures that might aid identification, especially of skeletal remains, severely decomposed bodies or those without any other identifying signs. The study's positive results enable various considerations to be made. Metal particles were released into tissues treated with osteosynthesis implants in all the bone sites examined (the main long bones). The finding of metal particles in bone areas where the implants had already been removed, even where there were no longer any radiographic signs of their application (e.g. the tibial malleolus), makes this observation particularly important in the forensic field for a number of reasons and especially with respect to identification. The release of metal particles into bone tissues could have various origins. Some authors have discussed biodegradation and corrosion of metal implants [1, 21], but release of microparticles could also take place during the actual fixation of the implant into the bone, as suggested by the results of this study, where the highest concentration of metal was found in the bone shafts closest to the osteosynthesis or fixation implants. It is also worth considering the possibility of migration of metal particles through the tissue, given that they were also found some distance away—in this study, up to 2.5 cm away from the implant. However, this theory seems less plausible, given the consistent absence of any signs of granuloma or cell reactions. Scanning electron microscope in association with energy dispersion spectrometry has recently been used in forensic medicine in different areas like gunshot residue analysis [25–27], dental comparison [28], diagnosis of work-related pulmonary diseases (silica particles) [29] and evidence of exogenous substances (sodium phosphate) [30]. In the demonstration of metal particles from osteosynthesis, SEM/EDS can be considered the most sensitive, accurate method for this type of investigation, but a preliminary examination under an optical microscope is recommended and can provide some important information. Finally, this study demonstrated that, following careful examination of the case history and any available intra-vitam radiological reports and the preliminary radiological screening of the cadaver or skeletal remains, bone samples must be taken not with metal tools (especially oscillating metal saws) but with specific tools (saws with ceramic blades) to ensure that any intra-bone particles encountered derive unequivocally from osteosynthesis devices implanted intra-vitam.

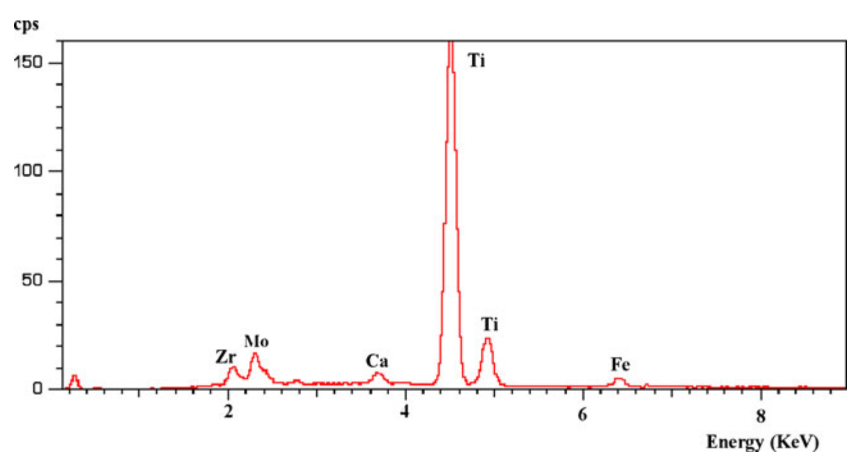


Fig. 3 EDS analysis of the metals found by SEM examination, identified as titanium (Ti) residues

Table 2 Overview of the optical and electron microscopy findings of the cases under investigation

Microscopical analysis			N° case and metallic debris localization									
			1		2		3		4		5	
			Place	Distance	Place	Distance	Place	Distance	Place	Distance	Place	Distance
Optical	Transmitted light		+	–	–	–	–	–	–	–	+	–
	Polarised light		+	–	+	–	–	–	–	–	+	–
SEM	Back-scattered electron		+	+	+	–	+	–	–	–	+	+
	EDS	Titanium	–	–	–	–	–	–	–	–	–	–
		Iron	+	+	–	–	–	–	–	–	+	+
		Cobalt	–	–	+	–	–	–	–	–	–	–
		Nickel	–	–	–	–	–	–	–	–	–	–
		Chrome	–	–	–	–	+	–	–	–	–	–

Microscopical analysis	N° case and metallic debris localization													
	6		7		8		9		10		11		12	
	Place	Distance	Place	Distance	Place	Distance	Place	Distance	Place	Distance	Place	Distance	Place	Distance
Optical	+	–	–	–	–	–	+	–	–	–	–	–	–	–
	+	–	+	–	–	–	+	–	–	–	+	–	+	–
SEM	+	–	+	–	–	–	+	+	+	–	+	+	–	–
	–	–	–	–	–	–	+	+	–	–	+	+	–	–
	+	–	+	–	–	–	+	+	–	–	+	+	–	–
	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	+	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Acknowledgements The authors wish to thank Mr. Agostino Rizzi, C.T.E.R. of the Istituto per la Dinamica dei Processi Ambientali (IDPA), CNR di Milano, working at the "Ardito Desio" Earth Sciences Department at the University of Milan, for his cooperation and tireless help with the SEM/EDS analyses.

References

1. Black J (1988) Does corrosion matter? J Bone Joint Surg 70-B:517–520
2. Zaffe D, Bertoldi C, Consolo U (2004) Accumulation of aluminium in lamellar bone after implantation of titanium plates, Ti-6Al-4V screws, hydroxyapatite granules. Biomaterials 25 (17):3837–3844
3. Dos Santos TI, De Oliveira PT, Rosa AL et al (2007) Histological and histomorphometric analysis of the bone–screw interface in the mandibular body after using a 2.0-mm miniplate system: an experimental study in dogs. J Oral Maxillofac Surg 65(11):2169–2175
4. Franchi M, Bacchelli B, Martini D et al (2004) A. Early detachment of titanium particles from various different surfaces of endosseous dental implants. Biomaterials 25(12):2239–2246
5. Franchi M, Orsini E, Martini D et al (2007) Destination of titanium particles detached from titanium plasma sprayed implants. Micron 38(6):618–625
6. Martini D, Fini M, Franchi M et al (2003) Detachment of titanium and fluorohydroxyapatite particles in unloaded endosseous implants. Biomaterials 24(7):1309–1316
7. Matthew R, Frame JW (1998) Ultrastructural analysis of metal particles released from stainless steel and titanium miniplate components in an animal model. J Oral Maxillofac Surg 56(1):45–50
8. Moberg LE, Nordenram A, Kjellman O (1989) Metal release from plates used in jaw fracture treatment. A pilot study. J Oral Maxillofac Surg 18(5):311–314
9. Nazzal A, Lozano-Calderón S, Jupiter JB et al (2006) A histologic analysis of the effects of stainless steel and titanium implants adjacent to tendons: an experimental rabbit study. J Hand Surg 31A(7):1123–1130
10. Gores RJ, Hayes CK, Unni KK (1989) Postmortem examination of six maxillary core-vent implants: report of a case. J Oral Maxillofac Surg 47(3):302–306
11. Hirai H, Okumura A, Goto M et al (2001) Histologic study of the bone adjacent to titanium bone screws used for mandibular fracture treatment. J Oral Maxillofac Surg 59(5):531–537
12. Rohrer MD, Bulard RA, Patterson MKJR (1995) Maxillary and mandibular titanium implants 1 year after surgery: histologic examination in a cadaver. Int J Oral Maxillofac Implants 10 (4):466–473
13. Torgersen S, Gjerdet NR, Erichsen ES et al (1995) Metal particles and tissue changes adjacent to miniplates. A retrieval study. Acta Odontol Scand 53(2):65–71
14. Bennett JL, Benedix DC (1999) Positive identification of cremains recovered from an automobile based on the presence of an internal fixation device. J Forensic Sci 44(6):1296–1298
15. Blau S, Robertson S, Johnstone M (2008) Disaster victim identification: new applications for postmortem computed tomography. J Forensic Sci 53(4):956–961
16. Kahana T, Ravioli JA, Urroz CL et al (1997) Radiographic identification of fragmentary human remains from a mass disaster. Am J Forensic Med Pathol 18(1):40–44
17. Simpson EK, James RA, Eitzen DA et al (2007) Role of orthopedic implants and bone morphology in the identification of human remains. J Forensic Sci 52(2):442–448
18. Bush MA, Bush PJ, Miller RG (2006) Detection and classification of composite resins in incinerated teeth for forensic purposes. J Forensic Sci 51(3):636–642
19. Bush MA, Miller RG, Norrlander AL et al (2008) Analytical survey of restorative resins by SEM/EDS and XRF: database for forensic purposes. J Forensic Sci 53(2):419–425
20. Acero J, Calderon J, Salmeron JI et al (1999) The behaviour of titanium as a biomaterial: microscopy study of plates and surrounding tissues in facial osteosynthesis. J Craniomaxillofac Surg 27(2):117–123
21. Jonas L, Fulda G, Radeck C et al (2001) Biodegradation of titanium implants after long-time insertion used for the treatment of fractured upper and lower jaws through osteosynthesis: element analysis by electron microscopy and EDX or EELS. Ultrastruct Pathol 25(5):375–383
22. Bouakaze C, Keyser C, Crubézy E, Montagnon D, Ludes B (2009) Pigment phenotype and biogeographical ancestry from ancient skeletal remains: inferences from multiplexed autosomal SNP analysis. Int J Legal Med 123:315–325
23. Benazzi S, Stansfield E, Dilani C, Gruppioni G (2009) Geometric morphometric methods for three-dimensional virtual reconstruction of a fragmented cranium: the case of Angelo Poliziano. Int J Legal Med 123:333–344
24. De Angelis D, Sala R, Cantatore A, Grandi M, Cattaneo C (2009) A new computer-assisted technique to aid personal identification. Int J Legal Med 123:351–356
25. Molina DK, Martinez M, Garcia J, DiMaio VJ (2007) Gunshot residue testing in suicides: part I: analysis by scanning electron microscopy with energy-dispersive X-ray. Am J Forensic Med Pathol 28:187–190
26. Pye K, Croft D (2007) Forensic analysis of soil and sediment traces by scanning electron microscopy and energy-dispersive X-ray analysis: an experimental investigation. Forensic Sci Int 165:52–63
27. Neri M, Turillazzi E, Riezzo I, Fineschi V (2007) The determination of firing distance applying a microscopic quantitative method and confocal laser scanning microscopy for detection of gunshot residue particles. Int J Legal Med 121:287–292
28. Fairgrieve SI (1994) SEM analysis of incinerated teeth as an aid to positive identification. J Forensic Sci 39:557–565
29. Fassina A, Corradin M, Murer B, Furlan C, Guolo A, Ventura L, Montisci M (2009) Detection of silica particles in lung tissue by environmental scanning electron microscopy. Inhal Toxicol 21:133–140
30. Viel G, Cecchetto G, Fabbri LD, Furlan C, Ferrara SD, Mentisci M (2009) Forensic application of ESEM and XRF-EDS techniques to a fatal case of sodium phosphate enema intoxication. Int J Legal Med 123:345–350