31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Multidisciplinary Approach for In-deep Assessment of Joint Prosthesis Failure

F. Tessarolo, I. Caola, F. Piccoli, P. Dorigotti, E. Demattè, M. Molinari, M. Malavolta, M. Barbareschi, P. Caciagli, G. Nollo *Member, IEEE*

***Abstract*—In spite of advancement in biomaterials and biomechanics, in development of new osteo-integrative materials and coatings, and in macro- micro- component design, a non negligible fraction of the implanted prosthesis fails before the expected lifetime. A prospective observational clinical study has been conducted to define and apply a set of experimental techniques to in-deep assess the failure of joint prosthesis. Microbiological, histological and micro-structural techniques were implemented to specifically address phenomena occurring at the tissue-implant interface. Results obtained from 27 cases of prosthetic joint failure are discussed in terms of sensitivity and specificity. A procedural flow-chart is finally proposed for the assessment of joint prosthesis failure.**

I. Introduction

Besides structural properties and design of prosthetic materials, interactions between implanted components and periprosthetic tissues have a fundamental role in the outcome of implanted devices. Although hi-tech materials and surface modification techniques are available for the production of arthroprosthetic components, about 8% and 4% of the orthopaedic surgical interventions deals with prosthetic revision of hip and knee following implant failure [1]. It is of utmost importance to characterize in-deep the phenomena at the tissue implant interface that brought to implant revision. The clinical protocol for treating the patient with a failed arthroprosthesis strongly depends on the failure mechanism. A primary distinction should be realized between septic and aseptic loosening which mainly addresses for one or two stage revision surgery. The largest amount of failures (>70%) is, however, related to non-infective events at tissue-prosthesis interface bringing to aseptic loosening [2]. Osteolysis, i.e. resorption of the bone in contact with the prosthetic component, is a complex inflammatory process which can be triggered by many different factors: traumatic impact, wear debris, lack of prosthetic primary stability, etc. The comprehension of the failure-inducing phenomena requires an analytical approach able to characterize both biological and biomaterials aspects. The literature on the subject lacks of an integrated approach for characterizing prosthetic failure [3]-[6], [9]-[10].

This work is aimed at defining, applying, and integrating a set of experimental analytical techniques able to characterize in details modifications occurring at the tissue-implant interface causing or concurring to the failure of the arthroprosthesis.

Manuscript received April 7, 2009. This work was supported in part by the "Fondazione Cassa di Risparmio di Trento e Rovereto", Grant 2006.

F. T. and G.N. are with the Interdepartmental Centre for Biotechnologies and Dept of Physics, University of Trento, 35100, Trento Italy (corresponding author to: +39 0461 882775; fax: +39 0461 883952; e-mail: tessaro@science.unitn.it).

I. C. is with the Microbiology and Virology laboratory at the Department of Medicine Laboratory, Azienda Provinciale per i Servizi Sanitari, 38100 Trento, Italy. (e-mail: iole.caola@apss.tn.it).

F. P. is with the Section of Electron Microscopy at the Department of Medicine Laboratory, Azienda Provinciale per i Servizi Sanitari, 38100 Trento, Italy. (e-mail: federico.piccoli@apss.tn.it).

P. C. is with the Department of Medicine Laboratory, Azienda Provinciale per i Servizi Sanitari, 38100 Trento, Italy. (e-mail: patrizio.caciagli@apss.tn.it).

P. D., E. D., M. M., and M. M. are with the Department of Othopaedics and Traumatology, Azienda Provinciale per i Servizi Sanitari, 38100 Trento, Italy. (e-mail: paolo.dorigotti@apss.tn.it).

M. B. is with the Department of Pathological Anatomy, Azienda Provinciale per i Servizi Sanitari, 38100 Trento, Italy. (e-mail: mattia.barbareschi@apss.tn.it).

II. Materials and Methods

The work has been conducted according to the following procedural steps to provide a complete set of information pertaining to patient conditions, prosthesis records, surgical intervention modalities, and sample details.

1) Ethics and patients: interrogation of the ethical committee, definition of inclusion criteria, definition of tools for sensitive data collection, management and storage.

2) Samples collection: definition of the minimal biological and prosthetic samples needed for analysis. Definition of sampling, preservation, transportation, and processing protocols.

3) Sample analysis: definition of analytical processing protocols, quantification or semi-quantification of test results.

4) Data analysis: correlation of experimental data to clinical diagnosis and formulation of technical output from biological and biomaterials findings.

A. Patients informed consent and recruitment

All consecutive surgical interventions for joint revision of hip, knee, and shoulder conduced at three surgical centre of the Department of Orthopaedics and Traumatology were considered for inclusion in the study in the period from May 2007 to January 2009. Following approval of the Hospital ethical committee, patients were recruited after informed consent. A "Surgical intervention data form" has been properly drafted to collect information about patients, clinical relevant data and explanted components as well as to

label and catalogue samples in an effective but anonymous way.

B. Samples collection

The following samples typologies were collected during revision surgery: synovial fluid (SF), tissue biopsies (TBs), periprosthetic material at the tissue-prosthesis interface (PM), and prosthetic components (PCs).

SF was collected by arthrocentesis before skin or articular pseudocapsule incision to avoid contamination. SF volume, according to availability, was divided in three aliquots for cultural and microscopic analysis and stored in liquid growth medium flasks and vials with anticoagulant (sodium citrate).

A set of 6 TBs was obtained including pseudocapsule, tissue at prosthesis-bone interface and inflammatory tissue (if present). Three samples were immersed in sterile saline for cultural test and three were formalin fixed for histology. Each TB was identified and processed separately.

PM was collected by swabbing and preserved in Amies transport medium till cultural test.

Each explanted PC (acetabular cup, liner, and femoral shaft for hip prosthesis; femoral component, tibial plate, and polyethylene meniscus for knee; and omeral shaft and glenoid for shoulder) was singularly identified and labelled. A macroscopic characterization was taken by photographic description and annotation of the main features (component breakage, macro-cracks, major wear). Any adherent tissue residual or acrylic cement was preserved and samples were decontaminated and fixed by 10% buffered formalin, and finally stored at 4°C till processing for microstructural characterization.

C. Microbiology

Microbiological test were adapted from previously proposed methods [3]-[5],[7] and integrated in order to enhance the sensitivity of cultural techniques.

Smears were obtained from TBs and PMs. Gram's staining was realized for microorganisms and leucocytes detection. Aerobic blood, chocolate, and anaerobic blood agar were inoculated with TBs and PMs. Inoculated plates were incubated for 5 days at 37°C. SF was cultured in liquid medium under aerobic and anaerobic conditions. TBs were also cultured in liquid medium for 15 days at 37°C; in case of microorganisms growth, subcultures on blood agar media were realized. Each isolated strain was biochemically identified and the antimicrobial sensitivity profile was obtained, Microscan, Siemens (Germany).

D. Histology

TBs for histology were paraffin embedded, thin sectioned and stained with eosin and hematoxylin (E&E). A semi-quantitative method for scoring the amount of polymorphonuclear leukocytes (PMNs), histiocytes, lymphocytes, and giant-cells in the tissue sections was adapted from Mirra et al. [8].

Similar methods for the semi-quantification of foreign bodies in periprosthetic tissue were applied [8] on permanent sections stained with Oil red O (OrO) (protocol adapted from [9]-[10]). The observation at the microscope in transmitted light of OrO stained section showed in red polyethylene (PE) wear debris. E&E stained sections showed black metallic fragments, and translucent ceramic debris. Perl's staining (specific for the detection of trivalent iron in tissues) was realized on consecutive additional sections to avoid misinterpretation of hemosiderin residuals. Debris of poly-methyl-methacrylate cement (PMMAC) was detected indirectly by the circular holes left in the tissue after the polymer dissolution during the histological diafanization process in xilol.

E. Microstructural characterization

Microstructural characterization was performed by means of Scanning Electron Microscopy (SEM), XL 30, FEG environmental-SEM, FEI-Philips (Netherlands) and Energy Dispersive X-rays Spectroscopy, (EDXS), EDAX (NJ, USA) on explanted PCs and on specifically realized thin tissue sections.

1) Prosthetic explanted components: Each explanted PC was passed from formalin solution to pure phosphate buffer for excessive aldehyde removal. Adequate resizing to fit SEM chamber was provided by using a high precision diamond circular saw, Micromet M, Remet (Italy). Samples were than dehydrated in ascending hydroalcolic solutions, dried in a vacuum desiccator and finally gold sputtered. External prosthetic surfaces, conceived to be in direct contact with bone (uncemented prosthesis) or PMMAC (cemented prosthesis), were imaged by SEM in high-vacuum mode by collecting secondary electrons (SED) and backscattered electrons (BSED) signals at a magnification of 50x, 500x, 1000x and 5000x. The elemental composition of the surface was obtained by acquiring spot EDXS spectra at 30 keV and search-matching the detected peaks on database. After surface characterization, a pre-defined part of the prosthetic component was isolated with the circular saw, included in epoxy resin, and sectioned to obtain a transversal representative section. The section of interest was than polished with sandpapers (400 to 4000 grit), gold sputtered and mounted on stub. A set of images was obtained in SED and BSED mode. Bi-dimensional EDXS semi-quantitative maps were obtained to evidence coatings and their compositions. The elemental composition of the bulk of the prosthesis was obtained by acquiring a spot EDXS spectrum at about 2 mm far from the prosthetic surface.

2) Tissue sections: One thin (5µm) section per histological sample was obtained from paraffin embedded biopsies. Sections were collected on glass slides, dewaxed in xilol, washed in pure ethanol, dried, gold sputtered and mounted on stubs for SEM and EDXS. BSED images were collected at a magnification varying from 100 and 10000

times. Bi-dimensional EDXS semi-quantitative maps were obtained in representative areas, previously imaged by optical microscopy on equivalent sections stained by E&E and OrO. Micro-morphology and elemental composition of wear debris were identified for the main particulate categories: PE, acrylic, metallic and ceramic debris.

III. Results

Twenty-seven consecutive cases of surgical joint revision (20 hips, 6 knees, 1 shoulder) were included in the study (patients mean age 68.9(35-87) years; male:female 10:17; mean implant duration 80.8(2-216) months; rheumatoid arthritis 1). Methods listed in Table I were challenged by computing sensitivity (SE) and specificity (SP) in identifying prosthetic joint infection (PJI). The clinical post-operative diagnosis was considered as reference. The study included 7 septic and 20 aseptic loosening. SE and SP of the investigated methods are reported in Table I.

Cultural methods identified 4 PJIs among 7 septic loosening. Causative microorganisms were *S. aureus* (2/4), *S. epidermidis* (1/4), and non-spore-forming aerobic gram-positive rods (1/4). Positive cultures were obtained from SF (4/4), TB (4/4) and PM (1/4). Gram staining has no SE in detecting microorganism in TB and PM. Histological method presented higher SE for revealing PJIs, but failed to correctly diagnose aseptic loosening in a patient affected by rheumatoid arthritis.

Histological semi-quantification of wear debris revealed a high content (score 3+) of intra-tissue fragments in 12 aseptic cases. The integration of histological images with SEM and EDXS data on tissue sections allowed to deeply characterize the nature of the fragments showing that 4 cases presented high content of PE fragments, 6 of PMMA cement, 3 of titanium-titanium alloy, and 1 of ceramic (Figure 1).

The macroscopic description reported 1 major tribological failure for a PE acetabular liner. The micro-structural analysis of prosthesis surfaces and transversal sections evidenced 2 cases of coating detachment (Figure 2a-2c), and 6 cases of PMMA cement cracking and detachment from the prosthesis surface (Figure 2d).

TABLE I
Sensitivity and Specificity of Tested Methods for Identifying PJI

Method/s	Criterion	SE %	SP %
1) Cultures on solid medium	I) growth of the same micro organism in two or more samples	43	100
2) Cultures in liquid medium	II) growth of the same microorganism in two or more samples	57	100
3) Gram's staining for microorganisms	III) presence of the same microorganism in cultures	0	100
4) Gram's staining for leucocytes semi-quantification	IV) ≥5 leucocytes per microscopic field (1000x)	71	80
5) Hematoxylin and eosin staining of tissue section histopathology	V) ≥5 PMN leucocytes per microscopic field (400x)	100	95
1) + 2)	criterion I) or II)	57	100
1) + 2) + 4)	criterion I) or II) or IV)	100	80
1) + 2) + 5)	criterion I) or II) or V)	100	95
1) + 2) + 4) + 5)	criterion I) or II) or IV) or V)	100	80

SE = Sensitivity, SP = Specificity, PMN = polymorphonuclear leucocytes.
Note: antibiotic therapy was not discontinued before revision surgery.

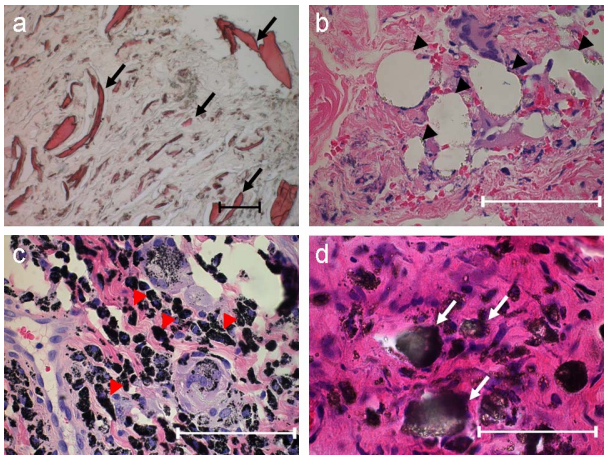

Fig. 1. Wear debris on histological tissue sections: a) polyethylene fragments from acetabular liner (black arrows) (OrO stain, original magnification 200x), b) holes in the tissue (black arrowheads) left by the xilol dissolution of PMMA cement debris (E&E stain, original magnification 630x), c) metallic debris (red arrowheads) from titanium acetabular cup (E&E stain, original magnification 630x), d) ceramic debris (white arrows) from failed alumina acetabular liner (E&E stain, original magnification 630x). Bar is 100 µm.

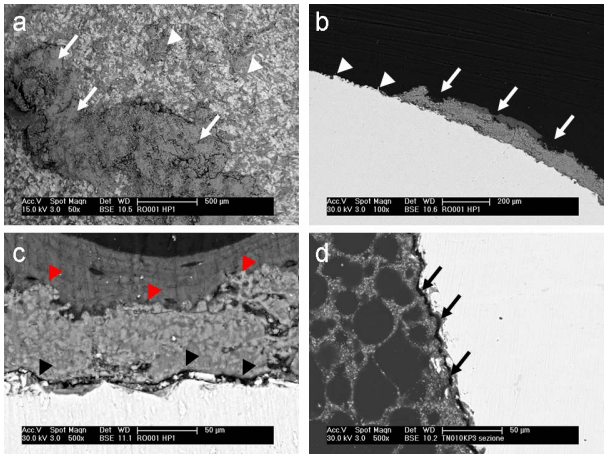

Fig. 2. Microstructural characterization of failed PCs imaged by SEM. Hydroxylapatite coating detachment (white arrows) from titanium alloy surface (white arrowheads) of a femoral shaft: a) *en-face* view, b) transversal section. c) good adhesion of periprosthetic bone (red arrowheads) and cracks propagating at the coating-prosthesis interface (black arrowheads). d) Detachment of PMMA cement (black arrows) from a tibial PC.

IV. Discussion

The integration of microbiological and histological methods is needed to identify PJIs. A number of TBs≥3 and PMs≥3 has to be collected intra-operatively for solid medium cultures and broth enrichment. The same microorganism has to be isolated in 2 or more samples to exclude contaminations. However, if the antibiotic therapy is not discontinued at least two week before intervention, cultural methods could reveal low sensitivity for detecting PJI. As previously reported [5], [8], we showed that an average number of PMNs≥5 on five independent microscopic fields at 500x magnification resulted as highly indicative for PJI. We confirmed that the semi-quantification of PMNs in perimplantar tissue sections is a sensitive method to support the diagnosis of PJI, but cultures are needed to identify the causative microorganism and its antibiotic resistance.

The integration of histological (Oil red O, Perls stains) and micro-structural characterization (SEM, EDXS) methods on properly processed tissue and prosthetic sample resulted in additional information for the in-deep characterization of aseptic failures dealing with material related phenomena at the tissue-bone interface.

According to experimental findings, a flowchart is proposed to screen among the most frequent failure mechanisms (Figure 3). All information and decisional criteria are available if the experimental protocol of sample collection and analysis presented in this work are followed. The etiology of aseptic failures here reported could give feedback for prosthesis defect, critical aspects, and potential ameliorations. Finally, to have an exhaustive comprehension of the failure phenomena, patient's history, hematic parameters, radiological evidences, surgical findings, and other investigative methods (i.e. marked leukocytes scintigraphy) should be considered.

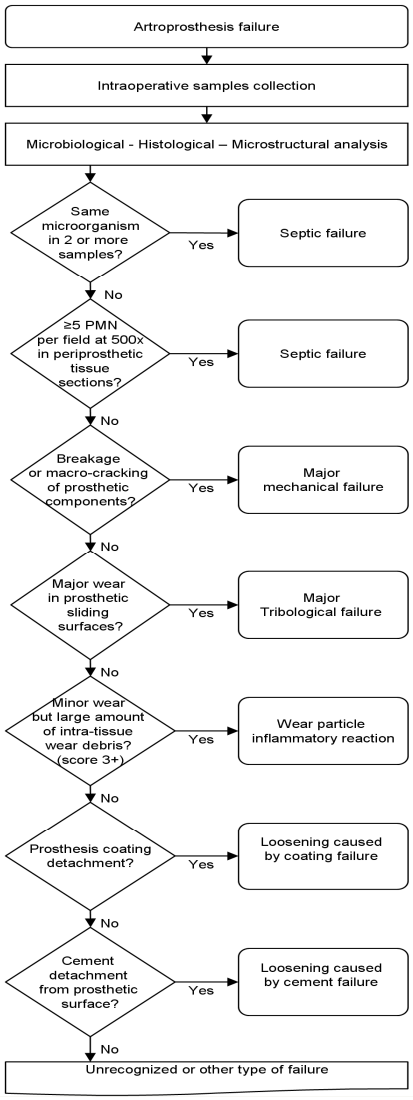

Fig. 3. Experimental flowchart for the assessment of prosthetic joint failure by a combined approach of microbiological, histological and microstructural methods.

Acknowledgment

Authors are grateful to surgical assistants and personnel of the surgical rooms at the Department of Orthopedics and Traumatology of the APSS for their contribution to biologic and prosthetic samples collection, preservation and labeling.

References

[1] M. Torre, E. Romanini, S. Palmieri, G. Zanoli, and G. Zapponi, "Registers of hip prosthesis interventions," *Bullettin of the Istituto Superiore di Sanità*; vol. 17, pp. 3-10, 2004.

[2] J. Kärrholm., G. Garellick., C. Rogmark, and P. Herberts, "*Swedish hip arthroplasty register - Annual Report 2007*," pp.1-132, 2007. Available: http://www.jru.orthop.gu.se/

[3] B. D. Brause, "Infection with prostheses in bones and joints,"in *Principles and practice of infectious diseases,* 4th ed. G. L. Mandell, J. E. Benett, R. Dolin Eds. New York: Churchill Livingstone, 1995, pp. 1051-1055.

[4] W. Zimmerli, A. Trampuz, and P. E. Ochsner, "Prosthetic-joint infections," *N Engl J Med*., vol. 351, pp. 1645-1654, 2004.

[5] R. Pandey, A. R. Berendt, and N. A. Athanasaou, "Histological and microbiological findings in non-infected and infected revision arthroplasty tissues," *Arch Orthop Trauma Surg*., vol. 120, pp. 570-574, 2000.

[6] S. Affatato, M. Spinelli, M. Zavalloni, C. Mazzega-Fabbro, M. Viceconti, "Tribology and total hip joint replacement: current concepts in mechanical simulation," *Med. Eng. Phys.,* vol. 30, pp. 1305-1317, 2008.

[7] F. Tessarolo, I. Caola, P. Caciagli, G. M. Guarrera, and G. Nollo, "Sterility and microbiological assessment in reusing single-use cardiac electrophysiology catheters," *Inf. Control Hosp. Epidemiol.,* vol. 27, pp.1385-1392, 2006.

[8] J. M. Mirra, H. C. Amstutz, M. Matos, and R. Gold, "The pathology of the joint tissues and its clinical relevance in prosthesis failure," *Clin. Orthop. Relat. Res.*, vol. 117, pp. 221-240, 1976.

[9] O. Rahbek, S. Kold, S. Overgaards, and K. Soballe, "Light microscopic identification and semiquantification of polyethylene particles in methylmethacrylate and paraffin-emebbed experimental bone implant specimens," *Journal of Microscopy*, vol. 218, pp. 225-232, 2005.

[10] T. Hansen, M. Otto, G. H. Buchhorn, D. Scharnweber, A. Gaumann, K. S. Delenk, A. Eckard, H. G. Willert., J. Kriegsmann, and J. Kikpatrick, "New aspect in the histological examination of polyethylene wear particles in failed total joint replacements," *Acta Histochemica*, vol. 104, pp. 263-269, 2002.