



International Congress of Science and Technology of Metallurgy and Materials, SAM –  
CONAMET 2014

## Influence of metalworking defect in knee cruciate ligament screws as a possible origin of infections.

Mario Spector<sup>a,\*</sup>, Guillermo García-Effron<sup>b</sup>, Leandro E Peretti<sup>a,c</sup>

<sup>a</sup>Universidad Tecnológica Nacional Facultad Reg. Paraná. Almafuerde 1033 Paraná E. Ríos Argentina.

<sup>b</sup>CONICET/Fac. Bioquímica y Ciencias Biológicas, Univ. Nac. del Litoral, Ciudad Univ. Ruta Nacional N° 168, km 472. Santa Fe. Argentina.

<sup>c</sup>Instituto de Desarrollo para la Industria Química, INTEC (Universidad Nacional del Litoral, CONICET), Güemes 3450, Santa Fe. Argentina.

### Abstract

A bone mucormycosis outbreak in a clinic of Paraná city after arthroscopic knee anterior cruciate ligament reconstruction forced to suspend these interventions. The main candidates as the infection source were the implanted screws. These screws were metallurgically analyzed. Microscopic defects like burrs and bending's were found within the screw's Allen heads. According to previous report Spector et al. (2013), these defects protect microorganisms and make difficult to eliminate them. The aim of this work was to demonstrate the implication of metal working defects in the titanium fixing screws as a reservoir of contaminated machining oil which could be the possible transmitting vehicle in the outbreak of bone mucormycosis. The genetic relatedness of the isolated strains was established, favoring the hypothesis that contaminated screws might be the infection sources.

© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Peer-review under responsibility of the Scientific Committee of SAM–CONAMET 2014

**Keywords:** Fungal infection, metallic prosthetics, surface defects

\* Corresponding author. Tel.: +54-343- 424-3054; fax: +54-343- 424-3054.  
E-mail address: [spectormario@gmail.com](mailto:spectormario@gmail.com)

## 1. Introduction

Arthroscopic knee anterior cruciate ligament reconstruction (ALR) is a routine procedure performed on the patient by implanting two screws (typically titanium), called transversal and interferential (Figure 1). Since 2005, Argentina suffers an outbreak of mucormycosis post ALR surgery including more than 40 cases (Makino et al, 2008). Different reports has considered the possibility of intraoperative seeding through contaminated surgical material (Burke 2002, Clara 2011), but only one of these warned about the probability of contaminated screws as the infection source (Chaves 2013)

The mucormycosis are a group of opportunistic mycoses caused by a group of fungi called Mucormycetes. Mucormycetes genres most commonly involved in human mycoses are *Rhizopus* spp., *Mucor* spp. and *Lichtheimia* spp. (Cheng et al., 2009; Gomes et al., 2011). The Mucormycetes share common features such as: high speed growth, ability to withstand wide temperature ranges, reproduce through spores produced in sporangia, have broad hyphae without septa and some genus have rhizoids that serve to anchor the substrate where they develop (as *Rhizopus* spp. and *Lichtheimia* spp.)

The clinical forms of mucormycosis are varied but always severe. In the three analyzed bone mucormycosis cases *Rhizopusmicrosporus* var. *rhizopodiformis* was isolated. This fungus is a human pathogen that requires special conditions to produce an infection. One of these conditions is the accidental inoculation by trauma or surgery using contaminated material. Bone mucormycosis produce bone necrosis manifested by severe pain, need for surgical bone resection, prosthetic bone replacement, temporary or permanent disability and in extremely severe cases can cause death (Figure 2).

Recently, Spector et al. demonstrated that metalworking defects in prostheses could protect Bacteria against the action of antibacterial drugs (Spector et al SAM / CONAMET 2013). Furthermore, it is known that machining oil is a medium in which fungi and bacteria can survive

The aim of this work was to report the implication of metal working defects in the titanium fixing screws as a reservoir of contaminated machining oil which could be the possible transmitting vehicle in the outbreak of bone mucormycosis caused by *Rhizopusmicrosporus* var. *rhizopodiformis*.

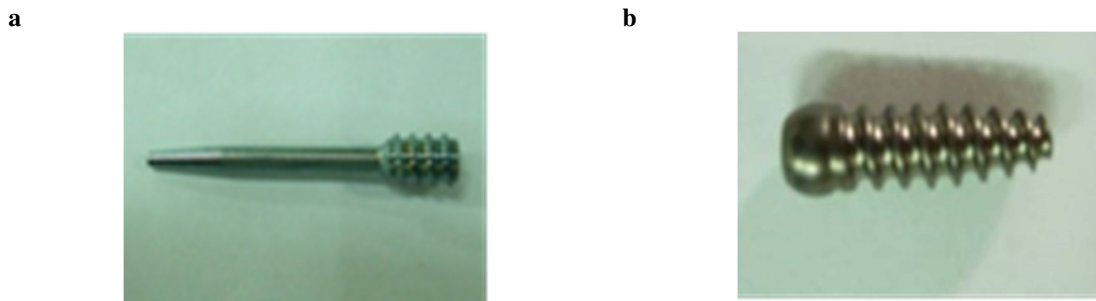


Fig. 1. (a) Transverse screw; (b) interferential screw.

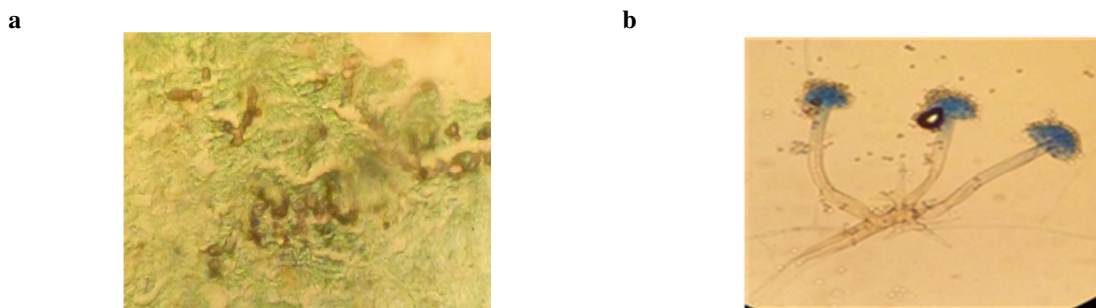


Fig. 2. (a) Hyphae of *Rhizopusmicrosporus* infecting human tissue (methenamine silver coloration 1000X); (b) photomicrograph at 400X of *R. microspores* where sporangia containing spores and characteristic rhizoids can be appreciated.

## 2. Materials and Methods

The samples used for this study included two sets of screws used in ALR surgery and extracted from infected patients, and four sets of new screws that could be purchased in the market (Figure 1).

### 2.1. Infected screws: magnifying glass inspection and metallography.

Using a magnifying glass (Motic, SMZ-168) a visual inspection of the internal and external surfaces of the screws was made (4x to 80x).

Metallographic samples were obtained following IRAM-IAS U 500-120 norms. The material was mounted in Bakelite and cupped using silicon as a fixative. It was sanded to 600 grit and finally polished with alumina grade II. Using a metallographic microscope (Tecnología Educativa, INV MET T) with a built camera (Moticam 3.0) metallographic samples were analyzed (100x and 200x). The analyzed sample was the head of the screw axially cut roughly in half, trying not to damage the metalworking imperfections.

### 2.2. Study of the genetic variability of isolates *R. microsporus* var. *rhizopodiformis*.

Random Amplification of Polymorphic DNA (RAPD) was used to analyze the genetic relatedness of the three *R. microsporus* var. *rhizopodiformis* isolated from the infections (Vágvölgyi et al. 2004). Moreover, 5 unrelated strains were included as non-related controls (1 isolate from France, 2 from other Argentinian cities and 2 environmental isolates). DNA from each of the strains was extracted by the phenol chloroform method adapted to Mucormycetes (Sambrook et al. 1998) and used as template in PCR reactions following the protocol published by Vágvölgyi et al. (2004). The results were evaluated with the Software PyElph, with which they were made dendograms to analyze patterns of size and intensity of the PCR fragments using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method (Vágvölgyi et al. 2004).

### 2.3. Feasibility machining oil is the source of infection

Sterile Metalworking oil was inoculated with different amounts of *R. microsporus* var. *rhizopodiformis* spores (reaching final concentrations of  $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$  and  $10^6$  spores ml oil<sup>-1</sup>). The inoculated oil samples were leaved at room temperature per 24 hours and then plated in Potatoe Dextrose Agar (Sigma Aldrich, Argentina). The plates were incubated at 35°C for 7 days and observed daily.

## 3. Results and Discussion

After visual inspection of the screws, it was found that screws external surfaces showed no deformities. Oppositely, metal working defects were found within the screw head (Allen hexagon) (Figure 3). Using metallography the metal working defects were more evident (Figure 4). The three *R. microsporus* isolates showed common genetic origin by RAPD analysis (Figure 5). These results demonstrate that the isolated fungi from the post ARL surgery wound infections have to have a common source. When the machining oil was studied as a possible source of screws contamination, it was found that the fungicidal capacity of the oil is low. It can inhibit grow of less than  $10^3$  spores per ml of oil (Figure 6).



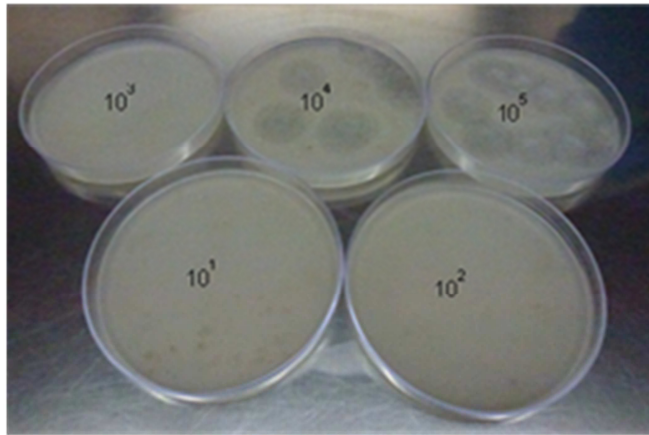


Fig. 6. PDA Petri dishes where counted inoculate contaminated machining oils were plated.

#### 4. Conclusions

The results presented here demonstrate that the metal working defects could act as a reservoir of the machining oil which can be contaminated with *R. microsporium* var. *rhizopodiformis*. Also, the presented fact reinforce the Spector et al. (2013) hypothesis that metal working defects (defined as burrs and embossing) could act as microorganism reservoir where chemical or physical decontamination procedures are inefficient.

#### References

- Burke, W., Zych, G. 2002. Fungal Infection Following Replacement of the Anterior Cruciate Ligament. A Case Report. *The Journal of Bone & Joint Surgery*. 84-A (3), 449-53
- Chavez, M. 2013. Dissertation: Arthroscopic Surgery Symposium of fungal infections associated with Arthroscopic Repair Ligament Crusaders, Paraná, Argentina.
- Cheng, V.C., Chan, J.F., Ngan, A.H., To, K.K., Leung, S.Y., Tsoi, H.W., Yam, W.C., Tai, J.W., Wong, S.S., Tse, H., Li, I.W., Lau, S.K., Woo, P.C., Leung, A.Y., Lie, A.K., Liang, R.H., Que, T.L., Ho, P.L., Yuen, K.Y., 2009. Outbreak of intestinal infection due to *Rhizopus microsporium*. *Journal of Clinical Microbiology* 47, 2834-2843.
- Clara, L., Almada, G., Salazar, E., Sánchez, M., Carbó, L., Barcan, L., Lizzi A. 2011. Mucormycosis in arthroscopic anterior cruciate ligament reconstruction. Infection control team. Hospital Italiano de Buenos Aires.
- Gomes, M.Z., Lewis, R.E., Kontoyiannis, D.P. 2011. Caused by mucormycosis mucormycetes unusual, non-*Rhizopus*, -*Mucor*, and -*Lichtheimia* species. *Clinical Microbiology Reviews* 24, 411-445.
- Mattsby-Baltzer, I., Sandin, M., Ahlstrom, B., Allenmark, S., Edebo, M., Falsen, E., Pedersen, K. 1989. Microbial Growth and Accumulation in Industrial Metal-Working Fluids, NILS RODIN. *American Society for Microbiology. Applied and Environmental Microbiology* 2681-268.
- Muscolo, D.L., Carbo, L., Aponte-Tinao, L.A., Ayerza, M.A., Makino, A. 2009. Massive bone loss from fungal infection after anterior cruciate ligament arthroscopic reconstruction. *Clinical Orthopaedics and Related Research*, 467 (9), 2420-5.
- Sambrook, J., Fritsch, E.F., Maniatis, T. 1998. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.
- Spector, M., Peretti, L., Salas, F., Romero, G., Iglesias, L. 2013. *Conducción Bacterial en Prótesis*. Congreso Sam Conamet 2013 Paper#T11c1, Misiones, Argentina.
- Vágvölgyi, C., Heinrich, H., Acs, K., Papp, T. 2004. Genetic variability in the species *Rhizopus stolonifer* assessed by random amplified polymorphic DNA analysis. *Antonie Van Leeuwenhoek* 876 (2), 181-8.