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Wear of implant materials — Polymer and metal wear particles — Isolation and characterization

Usure des matériaux d'implant — Particules d'usure des polymères et des métaux — Isolation, caractérisation et quantification

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 17853 was prepared by Technical Committee ISO/TC 150, *Implants for surgery*, Subcommittee SC 4, *Bone and joint replacements*.

This second edition cancels and replaces the first edition (ISO 17853:2003) and ISO 17853:2003/Cor.1:2004, which have been technically revised.

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Introduction

The biological responses to wear particles contribute to the failure of joint implants through bone resorption and consequent implant loosening. A standardized method of particle retrieval from the tissues followed by particle characterization is necessary for a uniform approach to wear particle effect investigations. The characterization of the particles generated from implants in joint simulators also provides valuable information on the wear properties and performance of the implant being studied.

In the protocols included in this International Standard, for isolation and characterization of particles from both tissues or test fluids from joint simulators, the particles are isolated and then dispersed using filtration or embedding in resin for scanning electron microscopy (SEM) or transmission electron microscopy (TEM) analysis. An alternative protocol for isolation and characterization of metal particles from implants tested in joint simulators has recently been developed in which the particles are deposited on to wafers for SEM analysis, without filtration or embedding; see Reference [1]. At the time of writing this International Standard, this alternative method had not been tested for isolation and characterization of particles from tissues and no direct comparison between the different methods is currently available. Therefore, the latter method has not been included in detail.

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Wear of implant materials — Polymer and metal wear particles — Isolation and characterization

1 Scope

This International Standard specifies methods for sampling wear particles generated by joint implants in humans and in joint simulators. It specifies the apparatus, reagents and test methods to isolate and characterize both polymer and metal wear particles from samples of tissue excised from around the joint implant, obtained at revision surgery or post mortem, and from samples of joint simulator test fluids. Some of these procedures could certainly be adapted for isolation and characterization of particles from human biological fluids (e.g. synovial fluid).

The methods given in this International Standard do not quantify the level of wear the implant produces; neither do they determine the amount of wear from any particular surface. This International Standard does not cover the biological effects of wear particles or provide a method for evaluation of biological safety.

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2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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2.1
polymer wear particles
particles generated from the wear of polymeric components of an implant

2.2
metal wear particles
particles and particulate corrosion products generated from the wear of metal components of an implant

2.3
ceramic wear particles
particles generated from the wear of ceramic components of an implant

3 Principle, reagents and apparatus

3.1 Principle

Particles of polymeric and metal wear are isolated from tissue samples and simulator lubricants by digestion. The yield of each particle species is then purified by eliminating any remaining organic debris.

NOTE The methods involved in polymer and metal particle isolation are different and are described in 4.2 and 4.3, respectively.

The particles are collected, and are characterized and counted (where applicable) using scanning electron microscopy (SEM) or transmission electron microscopy (TEM).

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3.2 Reagents

During the analysis, unless otherwise stated, use only reagents of recognised analytical grade and distilled water or water of equivalent purity.

All reagent solutions shall be filtered through a filter of 0,2 µm or smaller pore size prior to use, to avoid contamination of the sample by extraneous particles.

3.2.1 **Absolute ethanol**.

3.2.2 **Acetone**, 100 % or diluted with distilled water, with a volume fraction of 80 % acetone.

3.2.3 **Distilled water**.

3.2.4 **Fixative**, e.g. formalin, diluted with distilled water, with a volume fraction of 10 % formalin.

3.2.5 **Hydrochloric acid solution**, HCl, c = 0,01 mol/l.

3.2.6 **Isopropanol-water mixtures**, ρ = 0,96 g/cm^3 and ρ = 0,90 g/cm^3.

3.2.7 **Papain solution**, at 4,8 U/1,5 ml of 250 mM sodium phosphate buffer containing 25 mM ethylenediaminetetraacetic acid solution (EDTA), pH 7,4.

3.2.8 **Sodium phosphate buffer**, at 250 mM containing 25 mM of EDTA, pH 7,4.

3.2.9 **Proteinase K**, 2 g/ml of 50 mM tris(hydroxymethyl)-aminomethane-HCl (TRIS-HCl), pH 7,6.

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NOTE For particles isolated from joint simulator serum lubricant, the quantity should be adjusted depending on the serum percentage of the lubricant and initial serum volume from which particles are isolated. See 5.3.2.

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3.2.10 **Resin**, epoxy, such as EMbed 812.

3.2.11 **Sodium dodecyl sulfate (SDS)**, 2,5 g/100 ml solution in distilled water or 3 g/100 ml solution in 80 % acetone.

3.2.12 **Sodium hydroxide**, NaOH, solutions and pellets, c = 0,1 mol/l and 5 mol/l.

3.2.13 **Sucrose solutions**, ρ = 1,35 g/cm^3, 1,17 g/cm^3, 1,08 g/cm^3, 1,04 g/cm^3 and 1,02 g/cm^3.

3.2.14 **Tris-hydrochloride buffer**, TRIS-HCl, at 50 mM, pH 7,6.

3.3 Apparatus

All apparatus shall be cleaned and triple rinsed with distilled water previously filtered through a filter of 0,2 µm pore size (3.3.6) before use to remove any contaminating particles.

3.3.1 **Aluminium stub**.

3.3.2 **Balance**, with an accuracy of at least 0,1 mg.

3.3.3 **Carbon stickers**.

3.3.4 **Centrifuge tubes**, different sizes.

3.3.5 **Centrifuge**.

3.3.6 **Filters**, with a pore size of 0,2 µm for filtering reagents and distilled water.

3.3.7 **Filtration unit**.

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3.3.8 Formvar-coated copper grids, of 200 mesh size for TEM analysis.

3.3.9 Fourier Transform Infrared (FTIR) Spectroscope.

3.3.10 Heating plate.

3.3.11 Lint-free cloth.

3.3.12 Pipettes, micropipettes and tips.

3.3.13 Polarizing light microscope.

3.3.14 Polycarbonate membrane filters, of pore sizes 10 µm, 1 µm, 0,1 µm, 0,05 µm and 0,015 µm, for collecting particles.

3.3.15 Scanning electron microscope, SEM, with an energy dispersive X-ray analysis (EDXA) module.

3.3.16 Sterile Petri dishes, with lids.

3.3.17 Syringe, with wide-bore needle.

3.3.18 Teflon-glass potter tissue grinder.

3.3.19 Transmission electron microscope, TEM, with an energy dispersive X-ray analysis (EDXA) module.

3.3.20 Ultrasonic cell disrupter, equipped with a titanium microprobe.

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3.3.21 Ultrasonic bath.

3.3.22 Water bath, agitating temperature controlled.

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4 Methods of sampling and analysis of polymer and metal wear particles from tissue samples

4.1 Storage and preparation of samples

Store the tissue at –70 °C (or lower) in a freezer, or at room temperature in a fixative such as formalin (3.2.5), diluted with distilled water (3.2.4), with a volume fraction of 10 % formalin. Thaw the tissue, if applicable, and rinse it thoroughly in distilled water before continuing with the extraction method. Remove excess water from the rinsed tissue by blotting with a lint-free cloth (3.3.12).

Unfixed tissue should be handled under universal conditions.

The nature of surgical instruments used for sample retrieval should be recorded in case of contamination.

NOTE Sampling variability due to specimen origin can occur.

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4.2 Procedure for polymer particle isolation

4.2.1 Tissue digestion

There are many published methods for polyethylene particle isolation from periprosthetic tissues. The methods presented here are based on those of Campbell et al.[2], Tipper et al.[3] and Richards et al.[4].

Cut the tissue into smaller pieces using a scalpel and blade before digestion to speed up the digestion times. Extract the lipids from the minced tissue by placing into a 2:1 (volume ratio) chloroform:methanol mixture for 24 h or until the tissue sinks to the bottom of the container. Remove and rinse the tissue with PBS (3.2.8).

Add 5 mM NaOH (3.2.12) to the tissue in a ratio 10 ml of 5 mol/l NaOH to 1 g of tissue and leave to digest for a minimum of 24 h in an agitating water bath (3.3.22) at 65 °C. Digestion can be judged to be complete when no visible solid pieces of tissue remain in the suspension.

4.2.2 Purification of the polymer particle yield

4.2.2.1 General

The polymer particles can be purified from the digested tissue in a number of ways. Use one of the methods described in 4.2.2.2 or 4.2.2.3.

4.2.2.2 Purification of polymer particles by high-speed centrifugation

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This method enables all particle sizes to be collected from the nanometre-size range to several millimetres in length, enabling the total wear volume of particles to be isolated. Cool the digested tissue to 4 °C. Add an equal volume of ice-cold absolute ethanol (3.2.1). At this point, salts might precipitate. If this is the case, add ultrapure water until the salt dissolves. Incubate the solution at 4 °C overnight with stirring. Centrifuge the solution at 20 000 g for 2 h at 4 °C. Decant the supernatant liquid into a clean tube (3.3.4) and dilute with 400 ml of ultrapure water prior to filtration.

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4.2.2.3 Purification of polymer particles by ultracentrifugation

Place 2 ml of each sucrose solution (3.2.13) (ρ = 1,35 g/cm^3, 1,17 g/cm^3, 1,08 g/cm^3, 1,04 g/cm^3 and 1,02 g/cm^3) into centrifuge tubes (3.3.4) so that the tubes are roughly three-quarters full, and apply measured aliquots of the digested tissue suspension to the surface of the sucrose solution in each tube. Ultracentrifuge at 100 000 g for 3 h at 5 °C. Carefully collect the top layer into a sterile tube and dilute with distilled water at 65 °C to help dilute the residual sucrose. Ultrasonicate for 10 min to break up the agglomerated particles and then heat for 1 h at 80 °C to dissolve the sucrose.

Apply measured volumes of the suspension to two layers of isopropanol-water mixture (3.2.6) of densities 0,90 g/cm^3 and 0,96 g/cm^3 formed in ultracentrifuge tubes. Ultracentrifuge these at 100 000 g for 1 h at 20 °C. After removing the tubes from the ultracentrifuge rotor, a layer of white particles should be visible at the interface of the two layers. Remove this layer, containing the polyethylene particles, and place into a sterile tube using a fine-tipped glass pipette (3.3.12) inserted through the top isopropanol layer. Ultrasonicate for 10 min to break up any aggregates.

Different ultracentrifugation times and speeds may be used, provided that they have been demonstrated to give the same degree of separation and the results of the verification procedure have been documented.

NOTE 1 The first ultracentrifugation step serves to separate the lighter polyethylene wear particles from the heavier fractions. The second ultracentrifugation step purifies the polyethylene particle yield by putting it through a finer density gradient.

NOTE 2 This method might discriminate against the largest sizes of polyethylene generated, and consequently the total wear volume might not be isolated.

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4.3 Procedure for metal particle isolation

Due to the solubility of metals in strong acids and alkalis, an enzymatic digestion method needs to be used. The method below has been described by Catelas et al.[5] and is similar to the procedure developed earlier by the same authors for particle isolation from joint simulator lubricant {see Reference [6] (c.f. Clause 5)}, with only minor differences in the initial steps as well as in the enzyme concentrations to account for the use of tissue instead of serum lubricant.

NOTE 1 Being able to use the same procedure to isolate and characterize particles from tissues and joint simulator lubricant enables direct and accurate comparison of the isolated particles. This constitutes a significant advantage to this procedure.

a) Cut the tissue into small pieces using a scalpel and blade to speed up the digestion time. Resuspend several small pieces (about 2 mm × 2 mm × 2 mm) in 2 ml tubes.

NOTE 2 The tissue weight will depend on the overall wear noticed in the patient as well as the piece of tissue used for particle isolation (e.g. granuloma, capsule), but a minimum of 200 mg wet weight is recommended.

b) Wash four times for 2 min in sodium phosphate buffer (3.2.8), pH 7,4.

c) Resuspend the tissue pieces in 1 ml of sodium dodecyl sulfate (3.2.11) (2,5 g/100 ml solution in distilled water) and boil for 10 min. While boiling, homogenize the tissue pieces in solution using a Teflon-glass potter tissue grinder (3.3.18) every 2 min.

d) Cool at room temperature for 10 min.

e) Centrifuge the tubes at 16 000 g for 10 min.

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f) Wash once with 1 ml of acetone (3.2.2), diluted with distilled water with a volume fraction of 80 % acetone. Centrifuge at 16 000 g for 10 min.

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g) Wash three times with 1 ml of 250 mM sodium phosphate buffer containing 25 mM EDTA, pH 7,4. Centrifuge at 16 000 g for 10 min for each wash.

h) Sonicate in 1 ml of 250 mM sodium phosphate buffer containing 25 mM EDTA, pH 7,4, for 20 s to 25 s, using an ultrasonic cell disrupter equipped with a microprobe, or in a sonicating water bath for 30 min.

NOTE 3 Using the ultrasonic cell disrupter is more efficient, but an appropriate apparatus with a clean and undamaged/non-corroded probe tip is used to avoid potential titanium contamination from the probe tip.

i) Add 0,5 ml of 250 mM sodium phosphate buffer containing 25 mM EDTA, pH 7,4, and papain (3.2.7) (4,8 units per 1,5 ml of phosphate buffer). Incubate in an agitated water bath (3.3.22) for 24 h at 65 °C.

j) Centrifuge the tubes at 16 000 g for 10 min.

k) Carefully remove the liquid using a micropipette without touching the pellet at the bottom of the tubes.

l) Resuspend the pellet in 1 ml of sodium dodecyl sulfate (2,5 g/100 ml solution in distilled water).

m) Boil for 10 min.

n) Cool at room temperature for 10 min.

o) Centrifuge the tubes at 16 000 g for 10 min.

p) Carefully remove the liquid using a micropipette without touching the pellet at the bottom of the tubes.

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