INTERNATIONAL STANDARD

ISO 17853

First edition
2003-07-01

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

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

Reference number
ISO 17853:2003(E)

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Published in Switzerland

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Foreword

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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*.

Introduction

The biological responses to wear debris contribute to the failure of joint prostheses through bone resorption and consequent implant loosening. A standardized method of particle retrieval from the tissue, followed by debris characterization and quantification, is required for a uniform approach to debris response investigations. The examination of the debris generated from implants in joint simulators also provides valuable information on the wear properties and performance of the implant being studied.

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

1 Scope

This International Standard specifies methods for sampling wear debris generated by total joint prostheses in humans and in joint simulators. It specifies the apparatus, reagents and test methods to isolate, characterize and quantify both polymer and metal wear debris from samples of tissue excised from around the joint prosthesis, obtained at revision surgery or post mortem, and from samples of joint-simulator test fluids.

The method given in this International Standard does not quantify the level of wear the implant produces, nor does it determine the amount of wear from any particular surface. This International Standard does not cover the biological effect of wear debris or provide a method for evaluation of biological safety.

The method given in this International Standard is not applicable to the measurement of poly(methyl methacrylate) (PMMA) debris.

2 Terms and definitions

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

2.1
polymer wear debris
particles of ultra-high molecular weight polyethylene (UHMWPE) generated from the wear of polymeric components of a joint prosthesis

2.2
metal wear debris
particles and particulate corrosion products generated from the wear of metal components of a joint prosthesis

3 Methods of sampling and analysis of polymer and metal wear debris from tissue samples

3.1 Principle

Particles of polymeric and metal wear debris are released from tissue samples by digestion (see 3.5.1 and 3.6.1). 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 slightly different and are described in 3.5 and 3.6, respectively.

The particles are collected, and are characterized and counted using scanning electron microscopy (SEM) or transmission electron microscopy (TEM). The concentration of particles in the original tissue sample is then calculated.

3.2 Reagents

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

All reagent solutions shall be filtered through a filter of 0,2 μm pore size or less (3.3.4) prior to use, to avoid contamination of the sample by extraneous particles.

3.2.1 Sodium hydroxide solutions (NaOH), $c = 0{,}1$ mol/l and 5 mol/l.

3.2.2 Hydrochloric acid solution (HCl), $c = 0{,}01$ mol/l.

3.2.3 Sucrose solutions, $\rho = 1{,}35$ g/cm^3, 1,17 g/cm^3, 1,08 g/cm^3, 1,04 g/cm^3 and 1,02 g/cm^3.

3.2.4 Isopropanol-water mixtures, $\rho = 0{,}96$ g/cm^3 and $\rho = 0{,}90$ g/cm^3.

3.2.5 Distilled water.

3.2.6 Papain solution.

Mix 100 μm of pure papain, 3,26 mg of N-acetylcysteine, 9 ml of distilled water and 1 ml of phosphate buffer (3.2.7).

3.2.7 Phosphate buffer.

Dissolve 3,55 g Na_2HPO_4 and 3,45 g of NaH_2PO_4 in 100 ml of distilled water to obtain a solution of pH 6,5; add 0,744 g EDTA added after pH titration.

3.2.8 Ethylenediaminetetraacetic acid solution (EDTA).

Dissolve 250 g of EDTA salt in 1,75 l of distilled water.

3.2.9 Fixative, e.g. formalin.

3.2.10 High density polyethylene (HDPE) **powder** (particle length < 10 μm).

3.2.11 Titanium powder (particle length < 10 μm).

3.3 Apparatus

All apparatus shall be cleaned and triple-rinsed with distilled water which has been filtered through a filter of pore size 0,2 μm or less (3.3.4) before use, to remove any contaminant particles.

3.3.1 Lint-free cloth.

3.3.2 Ultracentrifuge.

3.3.3 Ultracentrifuge tubes.

3.3.4 Filters, with pore size of 0,2 μm or less for filtering reagents and distilled water.

3.3.5 Polycarbonate membrane filters, of pore sizes 0,1 μm and 0,02 μm, for collecting particles.

3.3.6 Agitating temperature-controlled **water bath**.

3.3.7 Pipettes, at least one of which is fine-tipped.

3.3.8 Petri dishes, with lids.

3.3.9 Ultrasonicator.

3.3.10 Polarizing light microscope.

3.3.11 Scanning electron microscope (SEM).

3.3.12 Transmission electron microscope (TEM).

3.3.13 Aluminium stub.

3.3.14 Carbon sticker.

3.3.15 Fourier transform infra-red (FTIR) **spectroscope**.

3.3.16 Syringe, with wide-bore needle.

3.3.17 Filtration unit.

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

3.4 Storage and preparation of samples

Store the tissue frozen in a freezer at −70 °C or in a fixative such as 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. Accurately measure the wet mass of the tissue and record the volume of the subsequently applied solutions to enable the calculation of the number of wear particles per gram of wet tissue (see 3.10).

Unfixed tissue should be handled under universal conditions.

Dry tissue may also be used, and the number of wear particles per gram of dry tissue calculated.

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

NOTE Sampling variability due to specimen origin may occur.

3.5 Procedure for polymer particle isolation

3.5.1 Tissue digestion

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 container. Remove and rinse the tissue.

Add 5 mol/l NaOH to the tissue in a ratio 10 ml of NaOH to 5 g tissue, and leave to digest for 1 h to 3 h in an agitating water bath at 65 °C. Digestion can be judged to be complete when no visible solid pieces of tissue remain in the suspension.

3.5.2 Purification of the polymer particle yield

Place sucrose solution ($\rho = 1{,}02$ g/cm^3) into ultracentrifuge tubes 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 2 °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 of densities 0,90 g/cm^3 and 0,96 g/cm^3 formed in the 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 UHMWPE particles, and place into a sterile tube using a fine-tipped glass pipette inserted through the top isopropanol layer. Ultrasonicate for 10 min to break up any aggregates.