#3728 - Posters

A Simple Method Of Isolation Of Polyethylene Debris From Periprosthetic Tissues Using Silicon Wafer Deposition

General Topics / Implants & Biomaterials

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Introduction
Several protocols of isolation of polyethylene (PE) debris from periprosthetic were presented so far. Most of them included deposition of particles on polycarbonate (PC) membrane filters, which can led to particle loss. Deposition of particles on silicon wafers allows to overcome this shortcoming, however the only protocol publicized so far is complicated and requires custom made equipment.
Objectives
To develop a simple method of separation, and deposition of PE particles on silicon wafers using standard laboratory equipment
Methods
PREPARATION OF SAMPLES : Fragments of periprosthetic tissues were harvested from 10 patients undergoing revisions of total hip replacements with metal on polyethylene bearing. Small fragments (approx. 300 mg wet weight) were freeze-dryed and delipidated in methanol and chloroform. Tissue was digested at room temperature in 10 ml of concentrated (65%) nitric acid for 48 hours. Samples were neutralized using 30 ml of methanol (such mixture has a density lower than PE), and centrifuged at 3000g for 15 min. The supernatand was removed, and the pellet containing PE debris and indigested protein fragents was resuspended in 4 cm3 of a solution containing 5 Mol of urea and 2% of SDS. The sample was sonicated do disperse the particles and divided into two equal parts, which were transferred into two standart 5 ml polycarbonate tubes which were weighted with a stainless steel ball. One tube was then used for deposition of PE debris on silicon wafers, the other for filtration - based separation.

PREPARATION OF WAFERS : Conductive silicon wafers were cleaved into small rectangular fragments (11x5 mm) and cleared ultrasonically in acetone. The central part of each wafer was coated with 20 ul of mussel glue suspension (BD CellTak), prepared according to manufacturer. Wafers were used for particle retrieval within 20 minutes after coating.

DEPOSITION ON SILICON WAFERS. One 5ml tube was placed into a standard 10 ml Polycarbonate tube (forming a nested tube), and a gradient of ultrapure water and 100% isopropanol was created in both tubes. A silicon wafer was placed on the opening of the small tube, with the coated side facing bottom (thus facing the suspension of PE particles). While

tubes were centrifuged at 3000g for 15 minutes, the PE particles floated from the bottom layer, and some of them adhered to the glue coated wafer. After removal of the top gradien tlayer the wafer was extracted from the tube. Wafers were then dried and examined using scanning electron microscopy (SEM)

FILTRATION BASED SEPARATION : Sample from the other 5ml tube was filtered through a PC membrane with 10 micrometer pores, and the filtrate was then transferred through a PC membrane with 0,1 micrometer pores. The membrane was then washed with 10 ml of methanol and dried.

SEM OBSERVATIONS : Tge membranes and wafers were sputter - coated with 10A of gold, and observed using SEM. The morphology, size and clumping of particles was evaluated.

Results

Observations in SEM showed good retrieval of samples using both methods, however deposition on silicon wafers demonstrated less clumping of particles, and allowed for retrieval of smaller debris, which were lost in the flitration method. The PC membranes also demonstrated more contamination with proteinous particles, compared to silicon wafers. Moreover the PE debris were uniformely distributed on the surface of the wafer, while the distribution was not homogenous, and showed a pattern corresponding to the grid shape of the filter holder.

Conclusions

The newly developed method allows for an eas, inexpensive and precise isolation of PE debris from periprosthetic tissues.The uniform distribution of the particles on the wafer allows for an easier analysis using SEM.