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REPORT ON IN-VITRO CELL ADHESION TO TITANMED DENTAL IMPLANTS

REF.: D.d.t. TITANMED S.r.l.

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Aim of the work

The aim of this work was the investigation of osteoblast-like cells adhesion to the surface of BRANE TU titanium dental implants, produced by Titanmed Srl.

Chemico-physical properties of materials surfaces play a fundamental role in determining the fate of bone contacting implant devices. Surface topography, in terms of kind of roughness, of porosity, affects cell adhesion, proliferation and differentiation: structural details of the cell body are the response to stimuli arising from the cell-surface contact. Based on these considerations, we evaluated adhesion and proliferation at different experimental time of osteoblast-like SaOS2 cells. SaOS-2 cell is a continuous cell line from human osteosarcoma, homogeneous and showing a stable phenotype, not fastidious and sharing many of the properties of not-transformed osteoblasts.

Materials

Tests were performed on 6 samples supplied by Titanmed Srl, fully packaged and sterile. In particular, as reported in the accompanying document, 6 implants BRANE TU 5 x 15 mm, lot 142/09.

Methods

Cell used for adhesion tests are SaOS-2 human osteosarcoma osteoblast-type cells, purchased from "Centro Substrati Cellulari dell'Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna". Cell adhesion tests have been conducted in accordance with the protocols listed in the international literature.

A suspension of $1.05\pm0.13 \times 10^5$ SaOS-2 cells (obtained by adding 2 ml of trypsin/EDTA solution to the monolayer inside a T75 Falcon flask) in 2.5 ml of McCoy's 5A medium, supplemented with 15% foetal calf serum, L-glutamine, penicillin, streptomycin and amphotericin B (all purchased from GIBCO, INVITROGEN Srl, San Giuliano Milanese) was introduced into sterile 12-well polystyrene culture plates (12-well multiwell plates, Cell Star, Greiner One™). At the same time, samples are extracted from their sterile package under a laminar flow cabinet and placed in

the multiwell. The culture plates are then placed in an incubator at 37°C, with 5% CO2 and 98% relative humidity. Samples were removed from the multiwell at a given experimental time (6, 24 and 72 h), delicately washed with Dulbecco's Phosphate Buffered Saline (DPBS, Gibco, INVITROGEN Srl), in order to remove any non-adhered cells and fixed using 4% glutaraldehyde in DPBS for at least 48 hours. Following fixation, cells were dehydrated by immersion for approx. 48 hours in each step of an alcohol-water series (the final step being in absolute ethanol, Fluka, Sigma-Aldrich Srl, Milan). Fixed and dehydrated samples were placed on sample holders on suitable conducting adhesive supports and coated with a thin layer of gold (Agar Sputter Coater). Observations by scanning electron microscope (SEM) were performed through a EVO MA 10 SEM. The operating parameters (working distance (WD), electron high tension (EHT), magnification (MAG), units of measurement, etc. Detector (Signal A) used over the course of observation are reported in the lower part of each image.

To provide a better understanding, the enclosed image presentation begins with two figures, obtained at 2000 and 5000 x of the bare implant surface, without adhering cells.

Results

Figures of the bare implant surface show the typical topography obtained through electrochemical treatment. The most distinctive feature is the presence of pore and "volcanoes", whose size ranges from a few tens of nanometers to a few micrometers. Obtained images immediately show that Titanmed BRANE TU implant surface support adhesion and growth of SaOS-2 cells. As a consequence, the first clear indication, that should never taken for granted without direct proof, is that the chemico-physical and topographic features of the present surface do not promote cytotoxic effects and do not prevent osteoblast cell adhesion. The observation, by the optical inverted microscope, of cells in the well, in areas not covered by the sample, allows to state that cells reached confluency in the adopted experimental time; moreover, these cells show the same morphology of those grown in the control well (that is the well containing just the cells, without implants).

Images obtained after 6 h contact (fig. 1- 10) show both well spread cells and cells that keep the globular shape typical of suspension, that is the initial cell shape at the moment of contact with the implant surface. The cell body, both for spread and globular cells, shows a high number of filopodia and pseudopodia, pointing everywhere and aiming at the exploration of the implant surface and at the connection with other cells. Filopodia are often seen entering the pores or holding the most elevated craters.

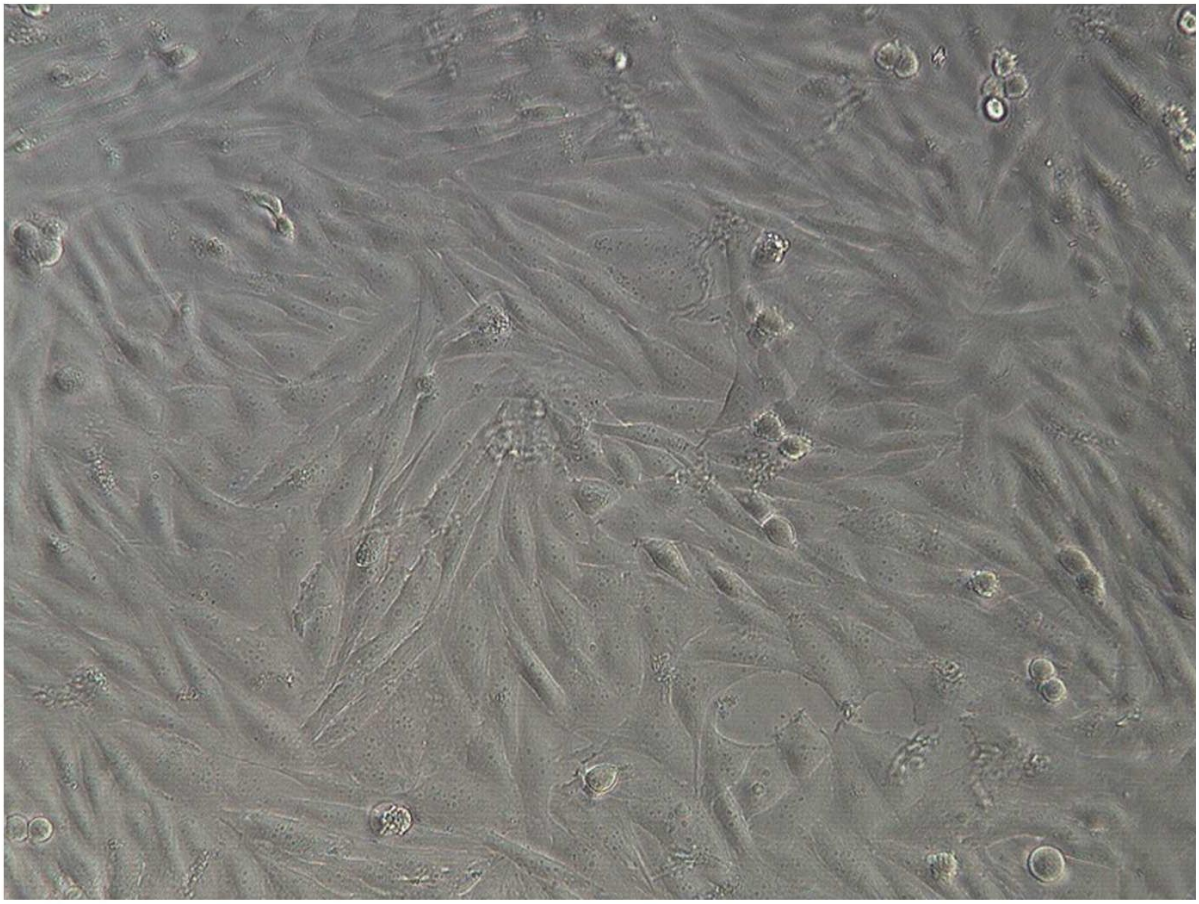
After 24 h (fig. 11- 23) images show an increasing number of well spread cells and a more pronounced surface colonization. Some globular cell is still observed, yet in a lower percentage as compared to 6 h. Surface topography is more and more hidden by the growing cell layer. Fig. 21, obtained at 35,000 x shows a filopodium entering into a pore, as if bringing the cell to anchor: the scale bar shows that the size of the filopodium and of the pore is of the order of a few tens of nanometers. After 72 h contact (fig. 24-34), most of the implant surface is covered by a conformal layer of cells, that hides pores and craters. Detected globular cells are actually lying on the thick cellular layer covering the surface. All images, especially those obtained at higher magnification, show an homogeneous and continuous cell overlayer, whose morphology is barely detectable, contrary to observation performed at 6 and 24 h. Shortly, these data show that, after the initial cell adhesion process, significant growth and proliferation of osteoblast cells on the implant surface occurred.

Conclusions

Obtained images show that the sandblasted surface of BRANE TU implants allows adhesion, colonization and extensive proliferation of SaoS-2 osteoblast cells. This result suggests that the implant surface is suitable for bone contact devices, and it does not contain toxic contaminants or residual, that for sure will not allow cell-surface interaction to proceed in the way documented by the present work.

IMPLANT TITANMED BRANE TU 5.0 X 15.0 mm Lot 142/09

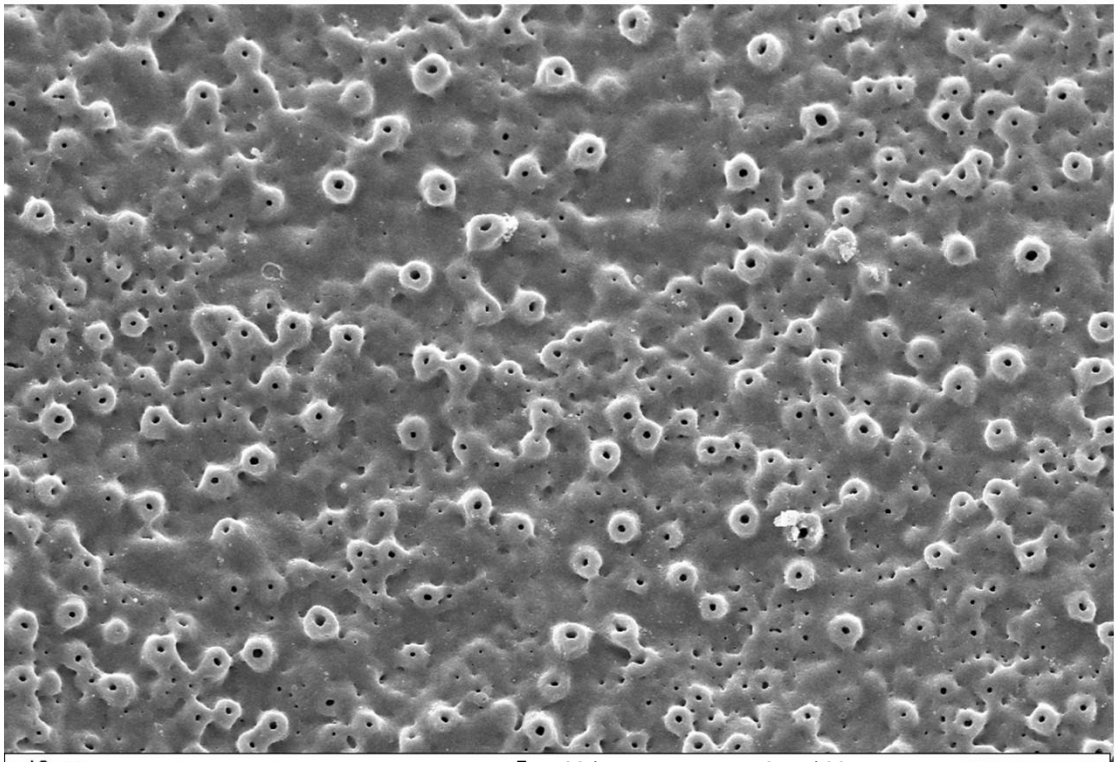
lmage of cells by optical microscope

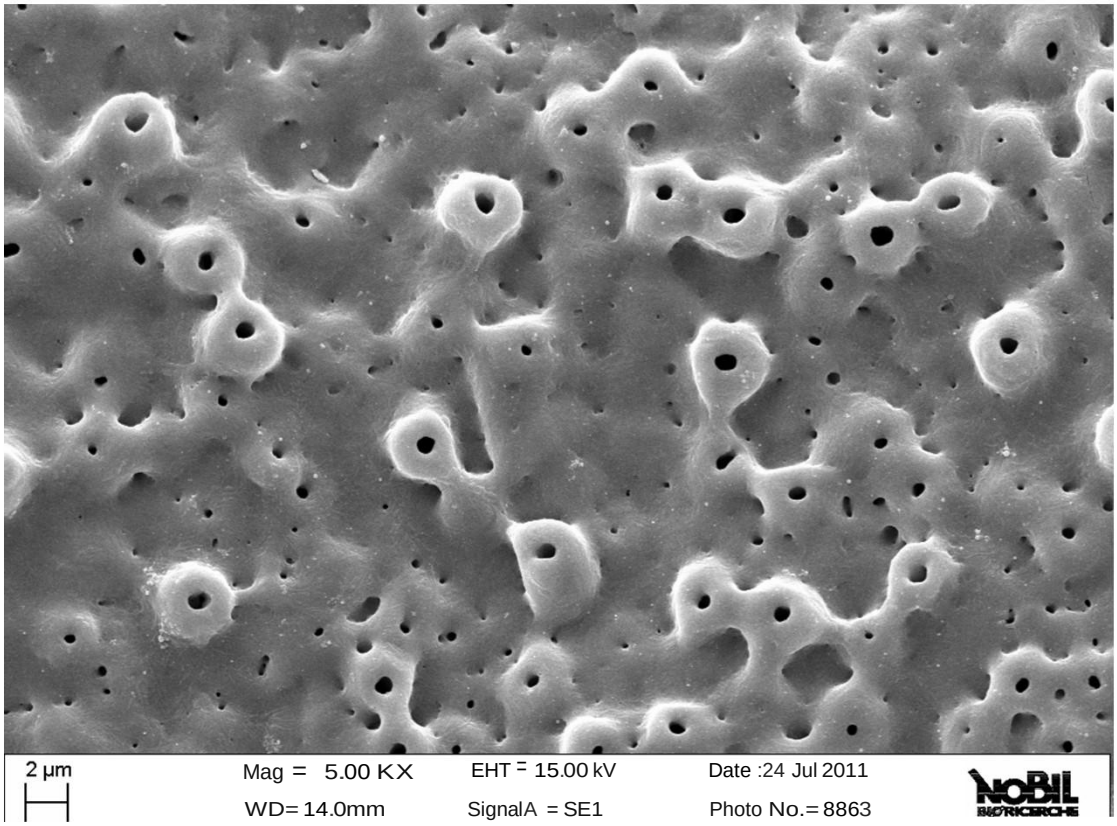


SAOS-2
CELLS ADHESION

TITANMED IMPLANT
BRANE TU 5.0 X 15.0 mm
Lot 142/09

IMPLANT BRANE TU 5.0 X 15.0 mm Lotto 142/09
SURFACE WITHOUT CELLS





6 HOURS

Fig. 1

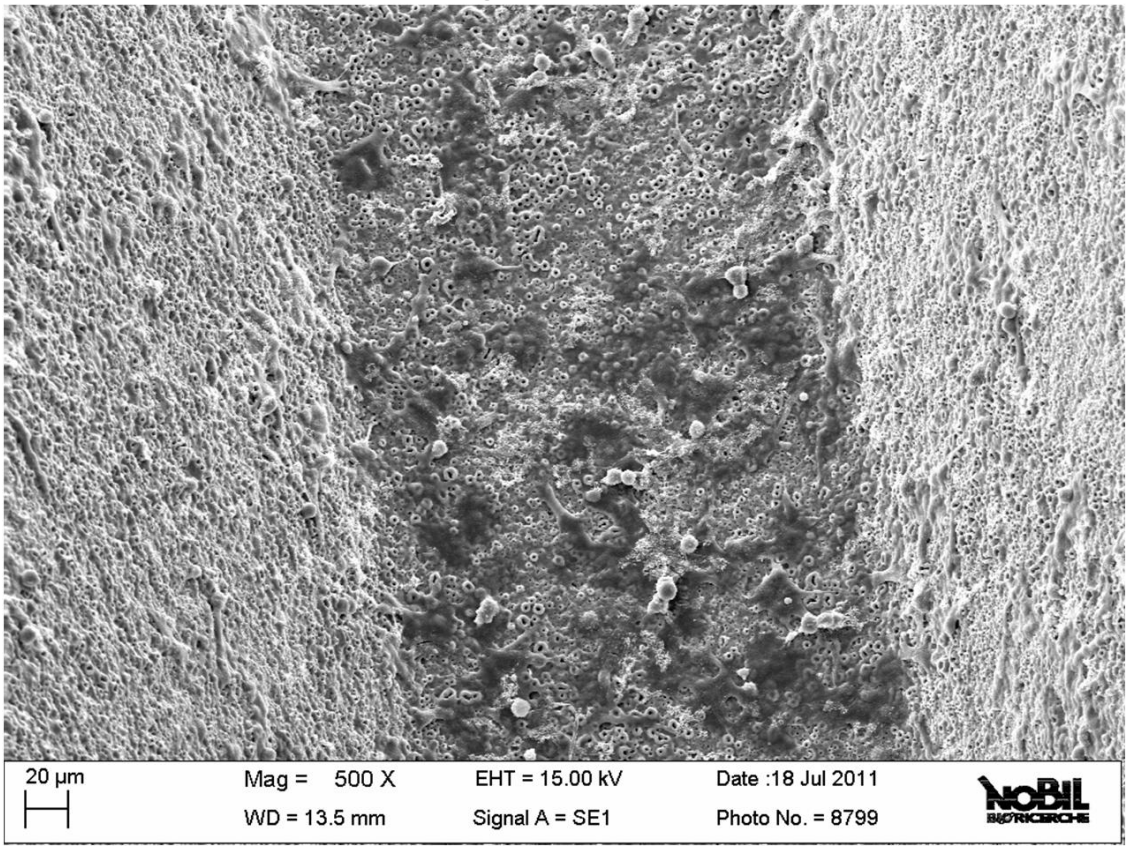
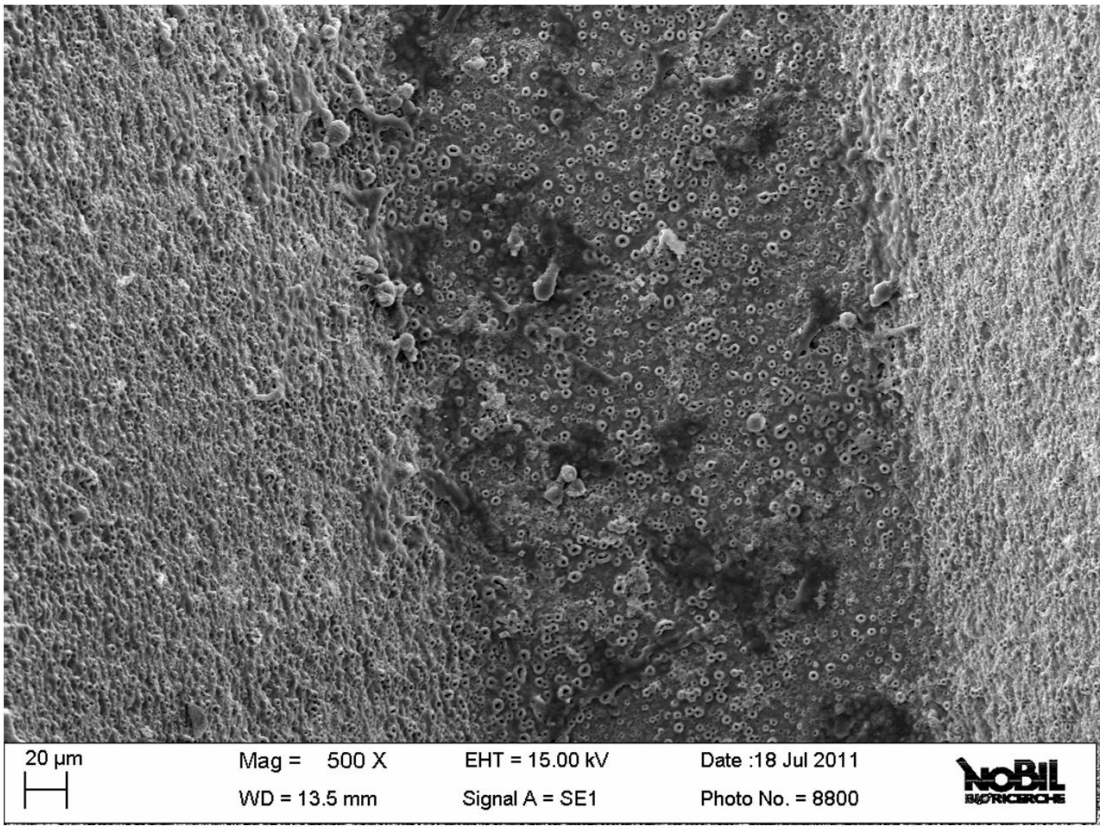


Fig. 2



6 HOURS

Fig. 3

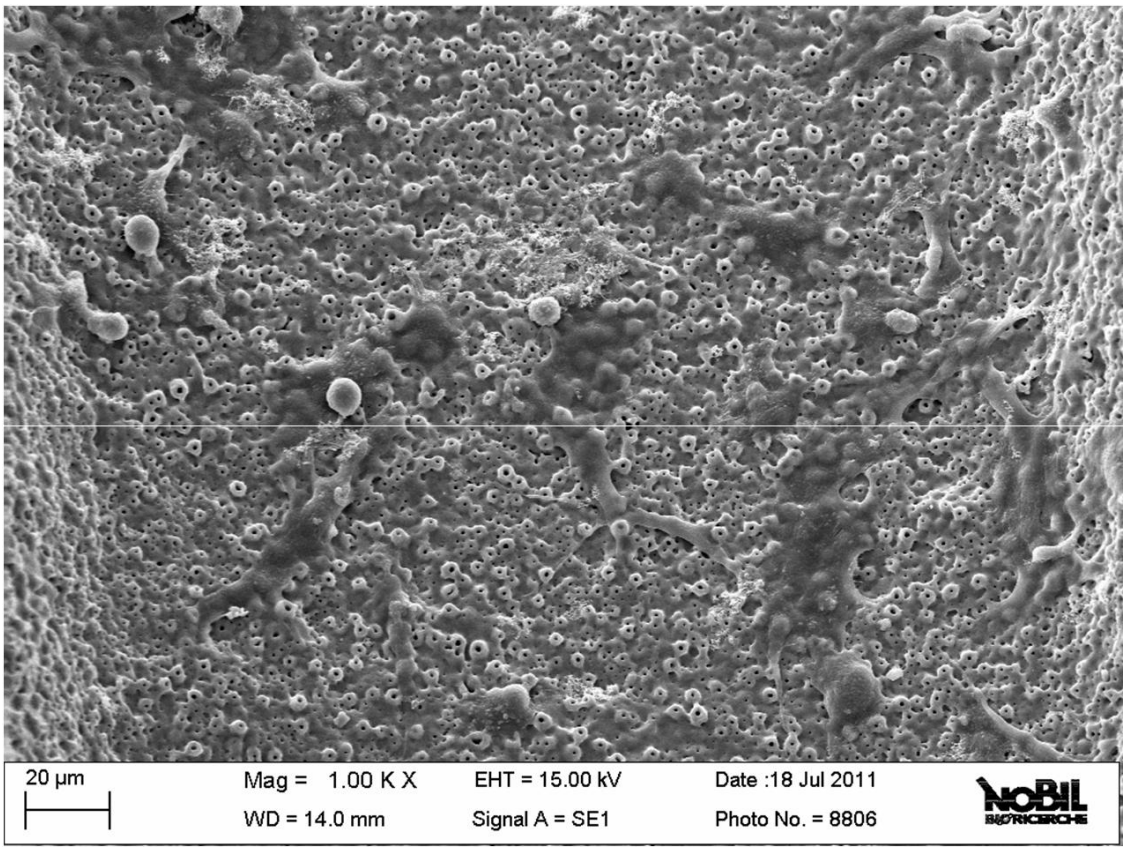
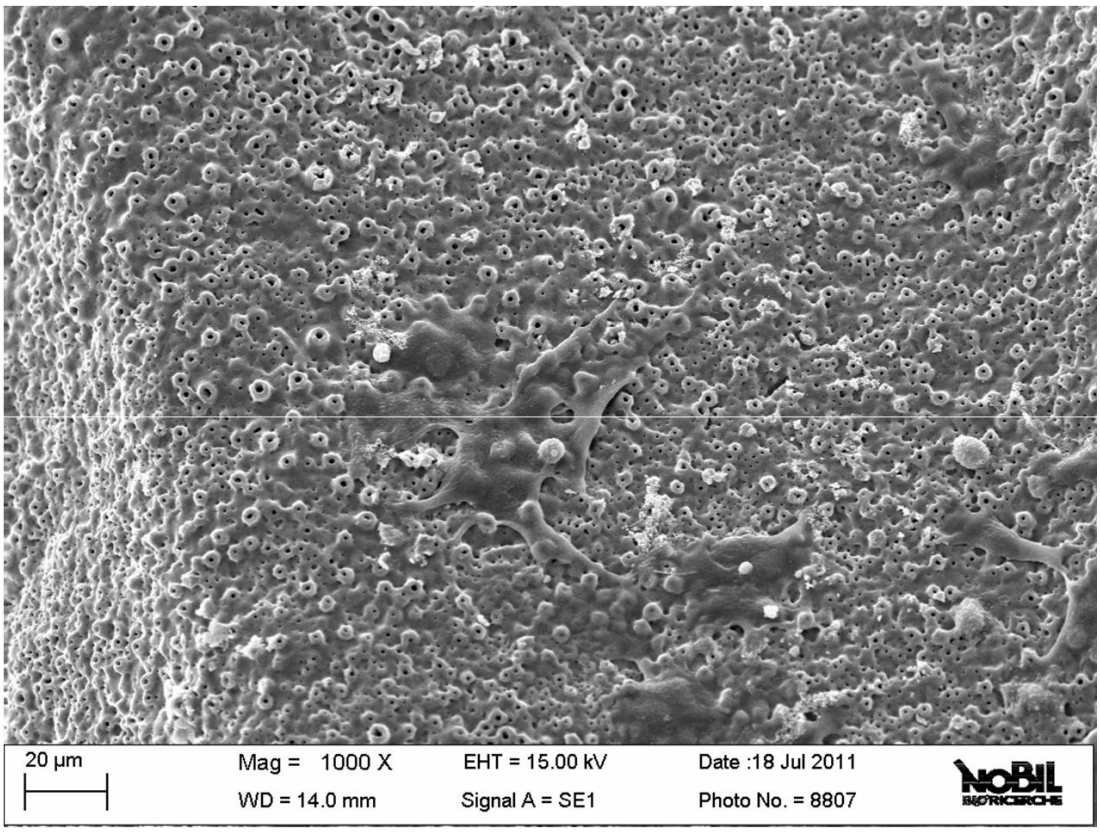
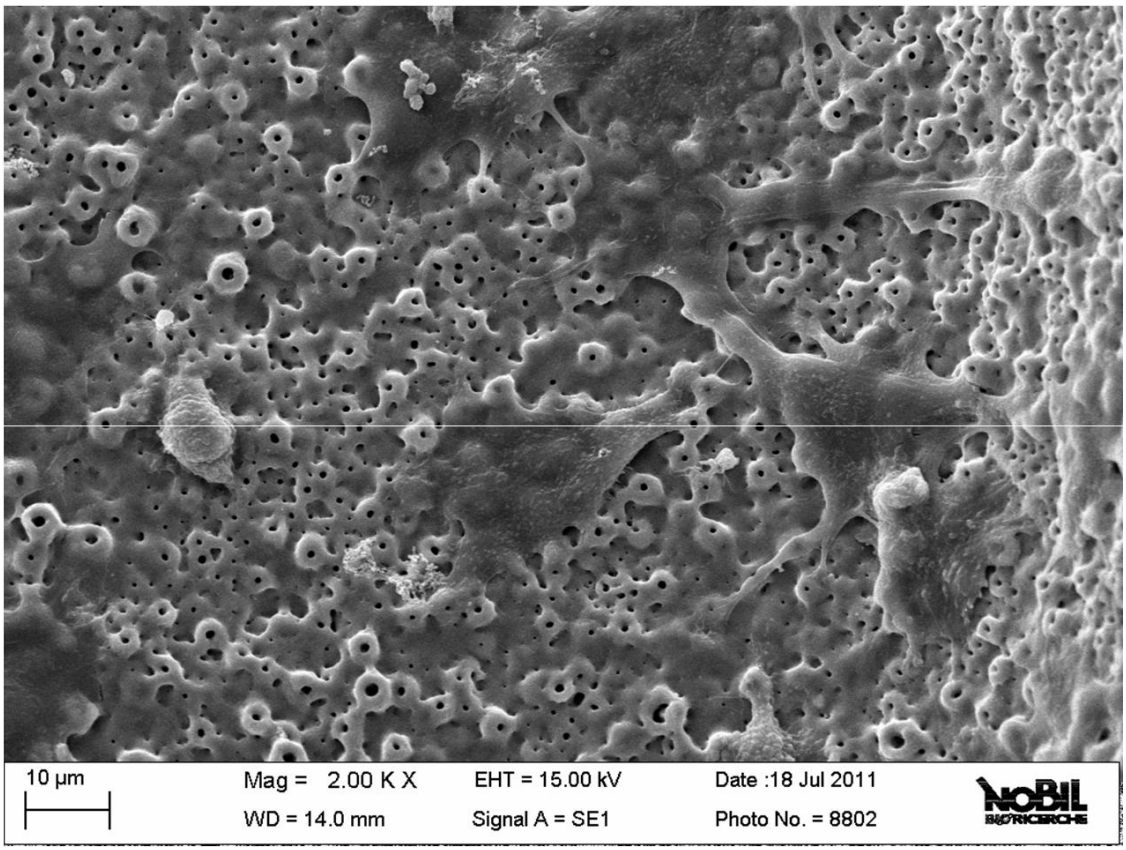


Fig. 4



6 HOURS

Fig. 5



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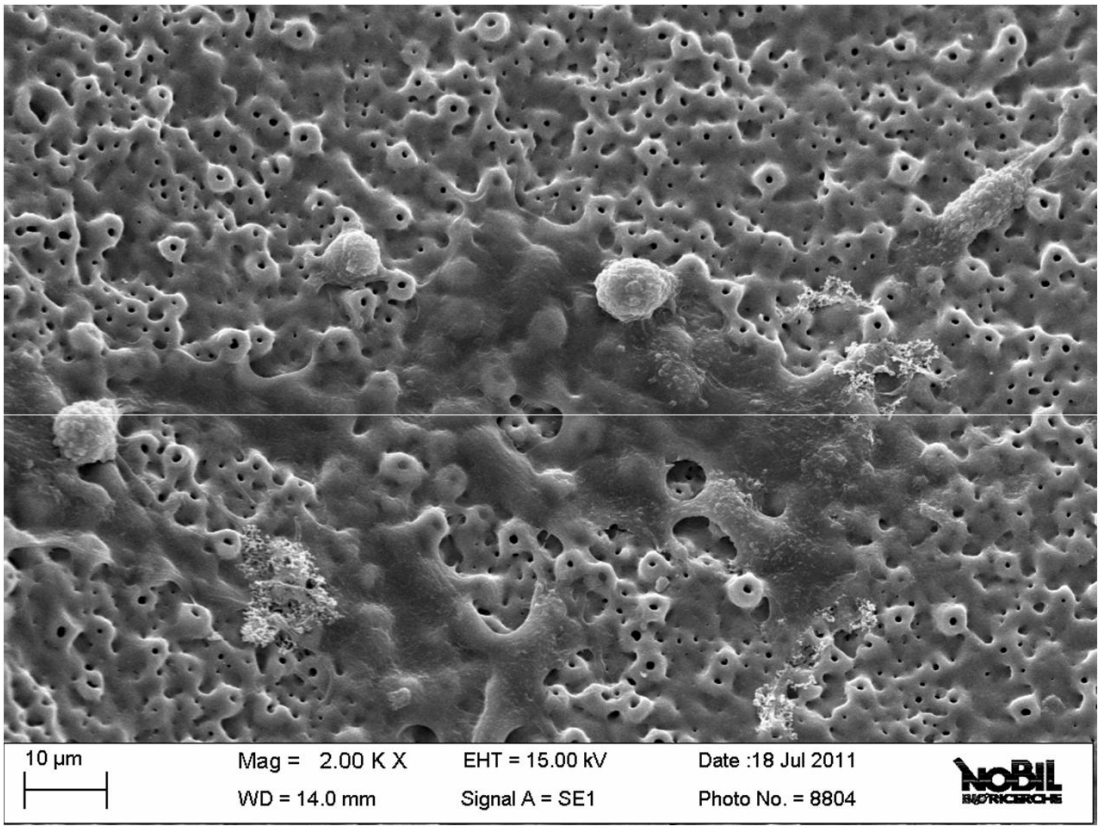

6 HOURS

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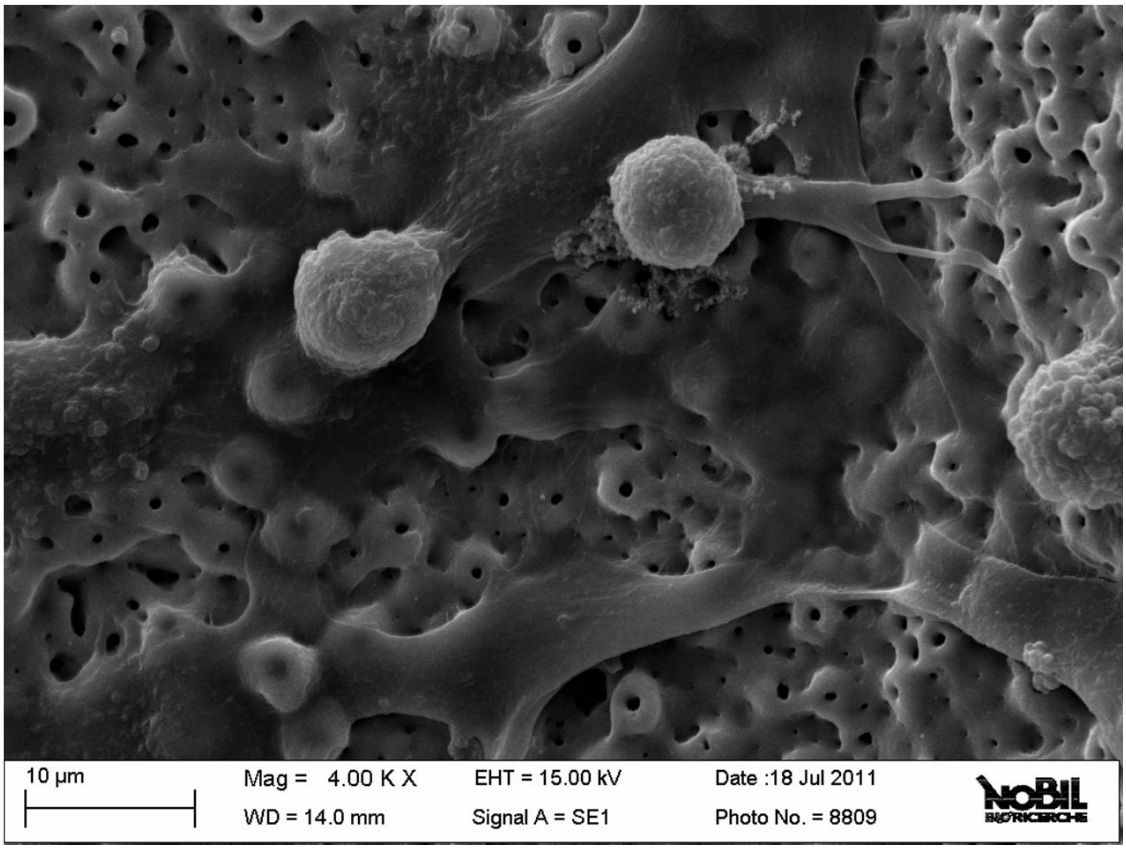

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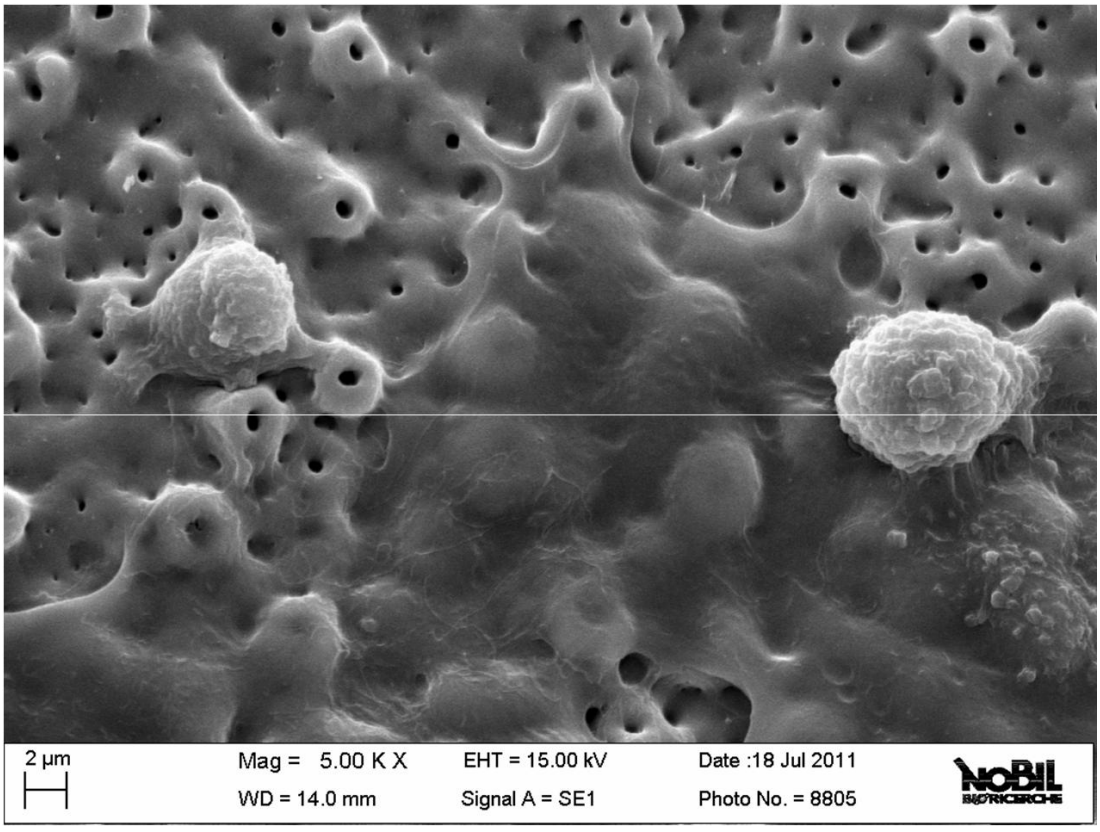

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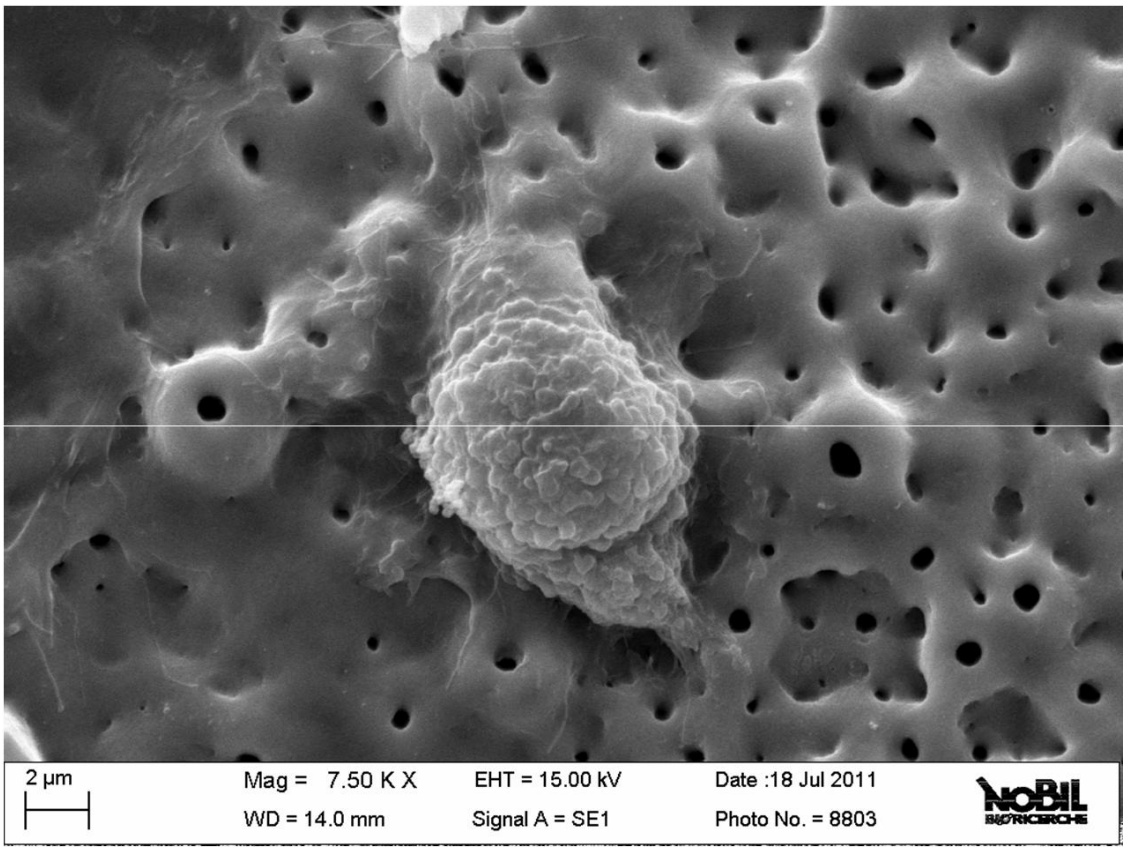
Fig. 9



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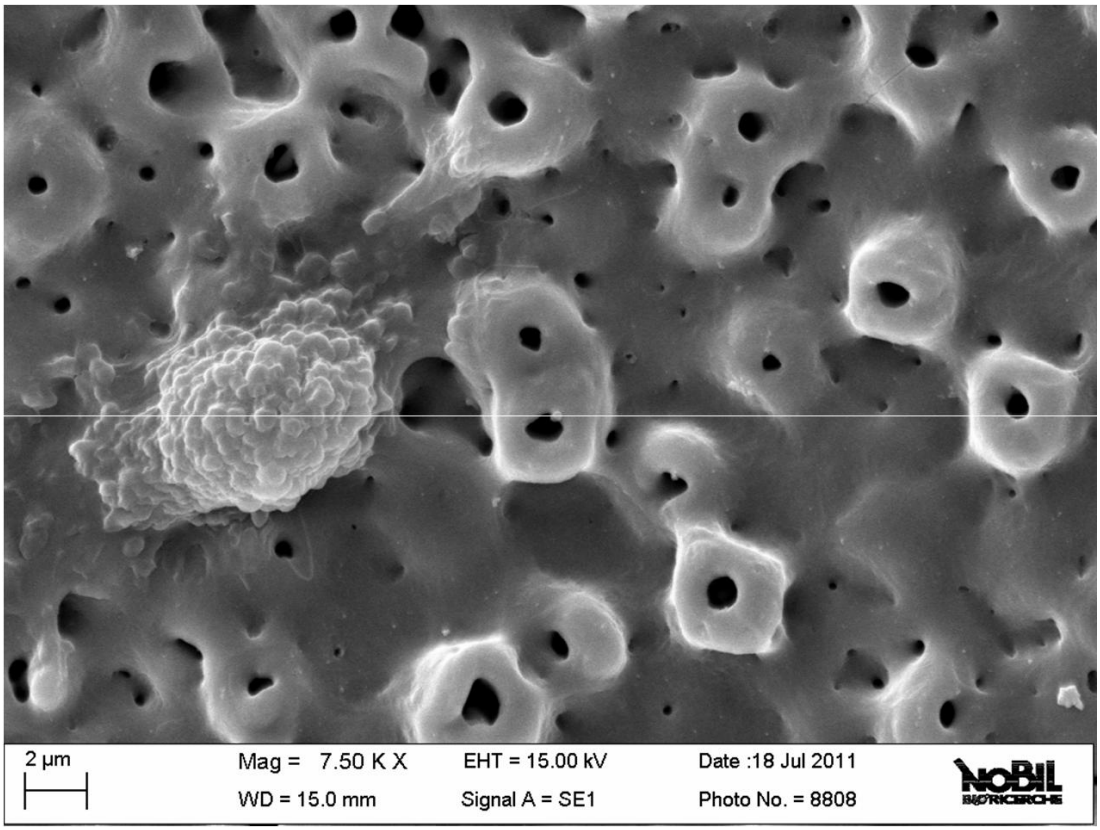

24 HOURS

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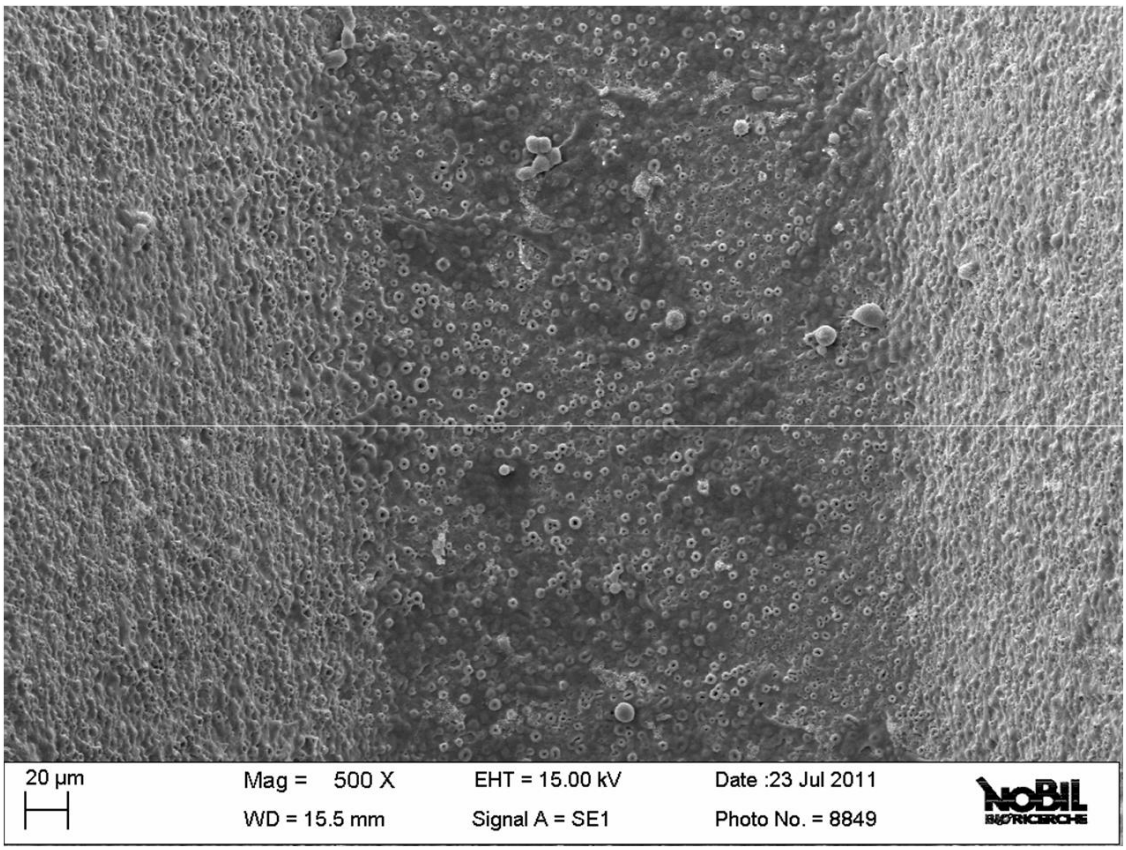

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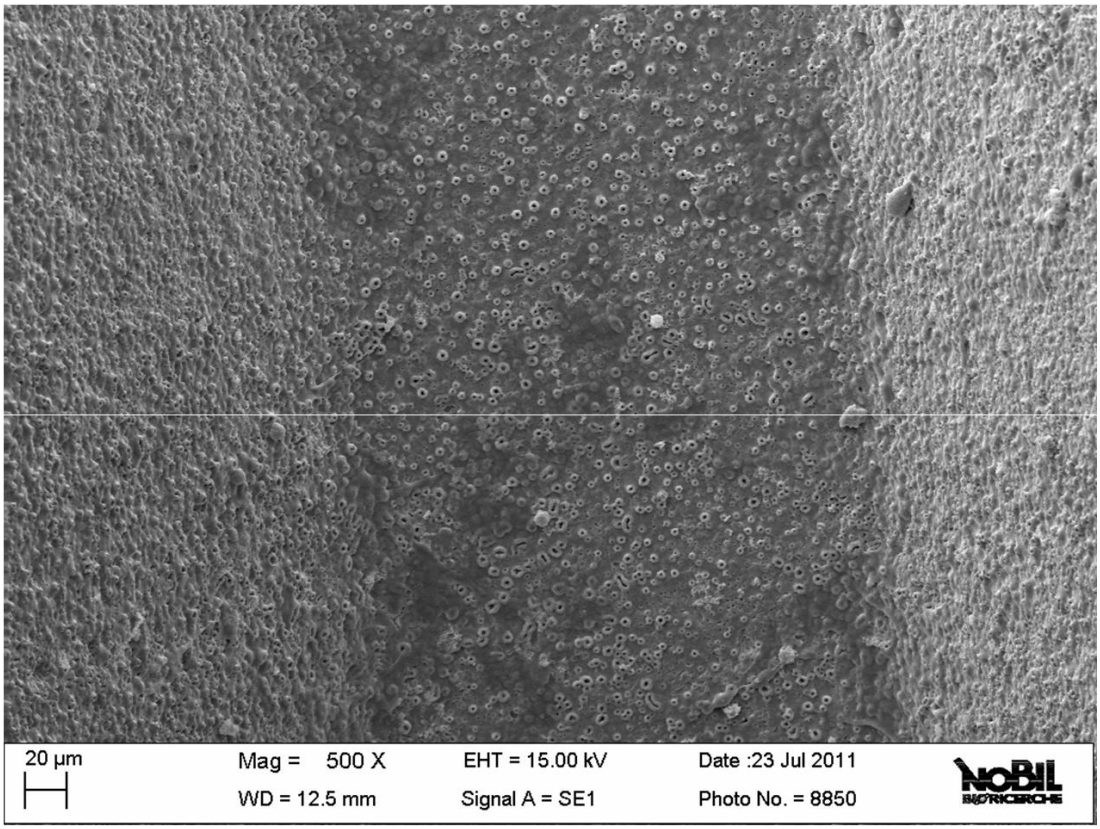

24 HOURS

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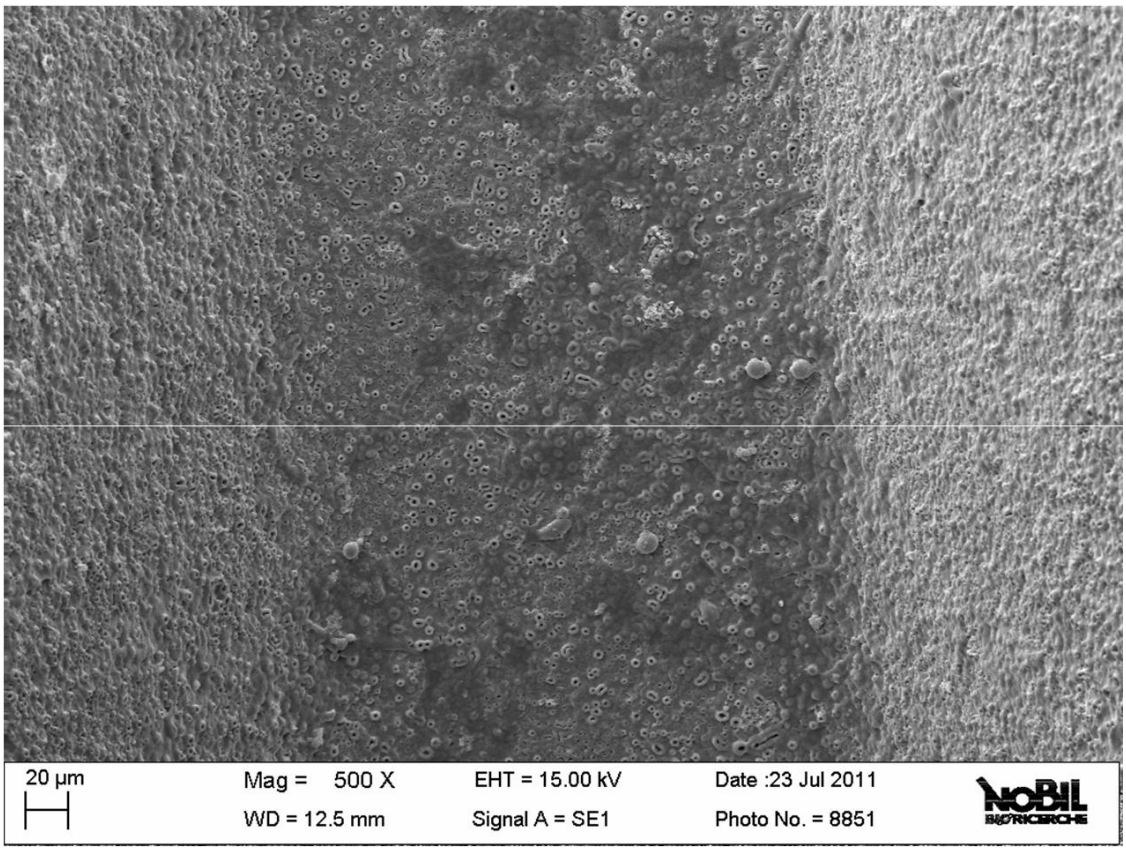

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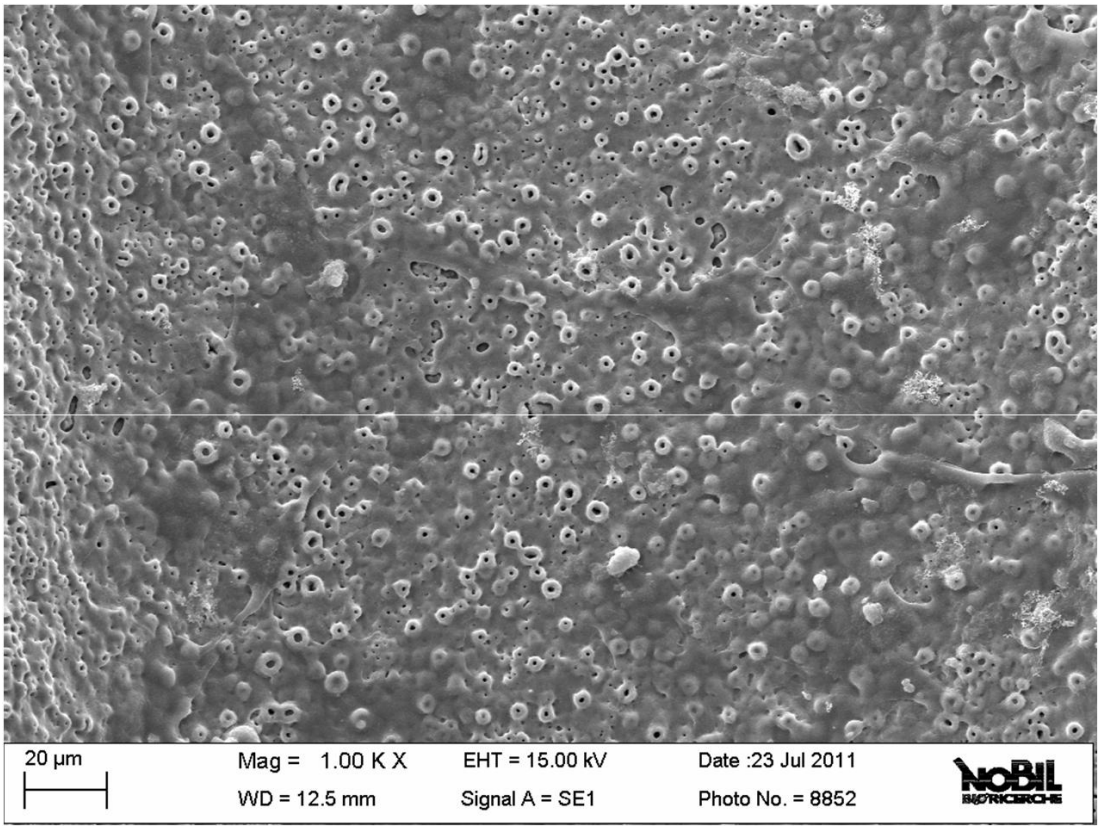
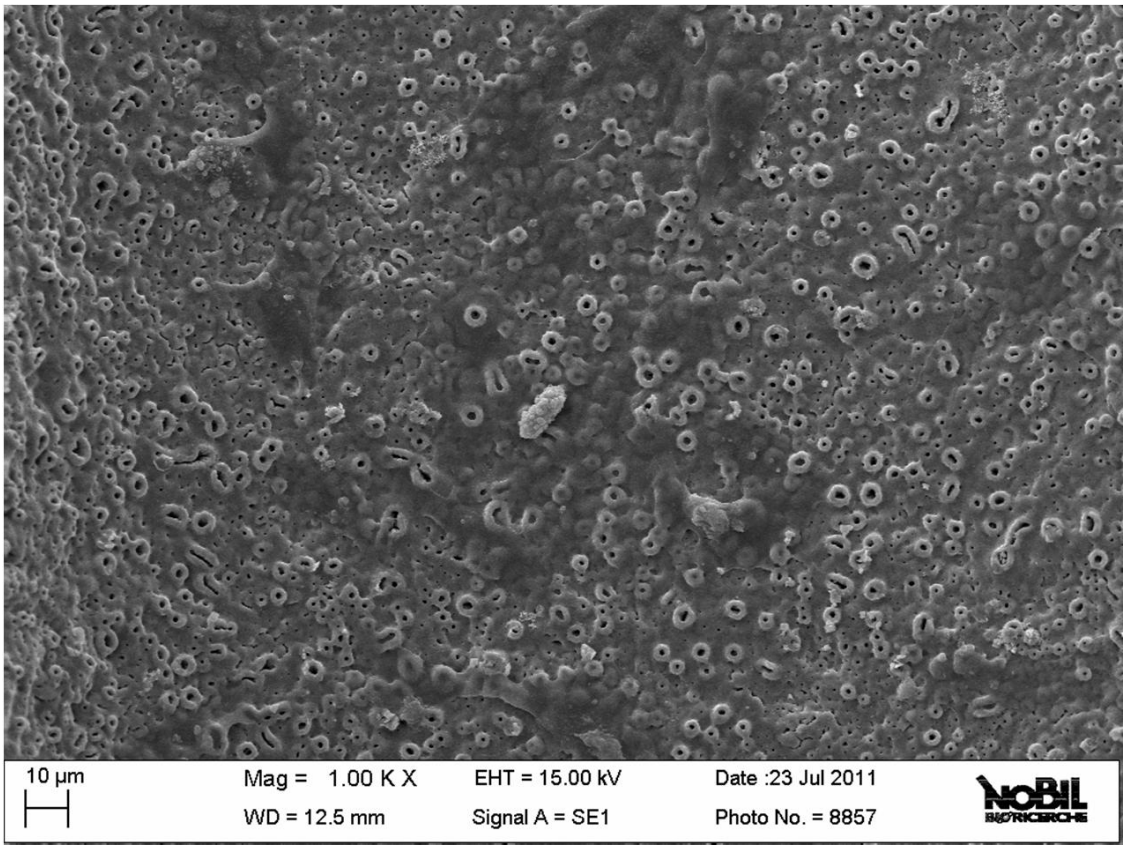


24 HOURS

Fig. 15



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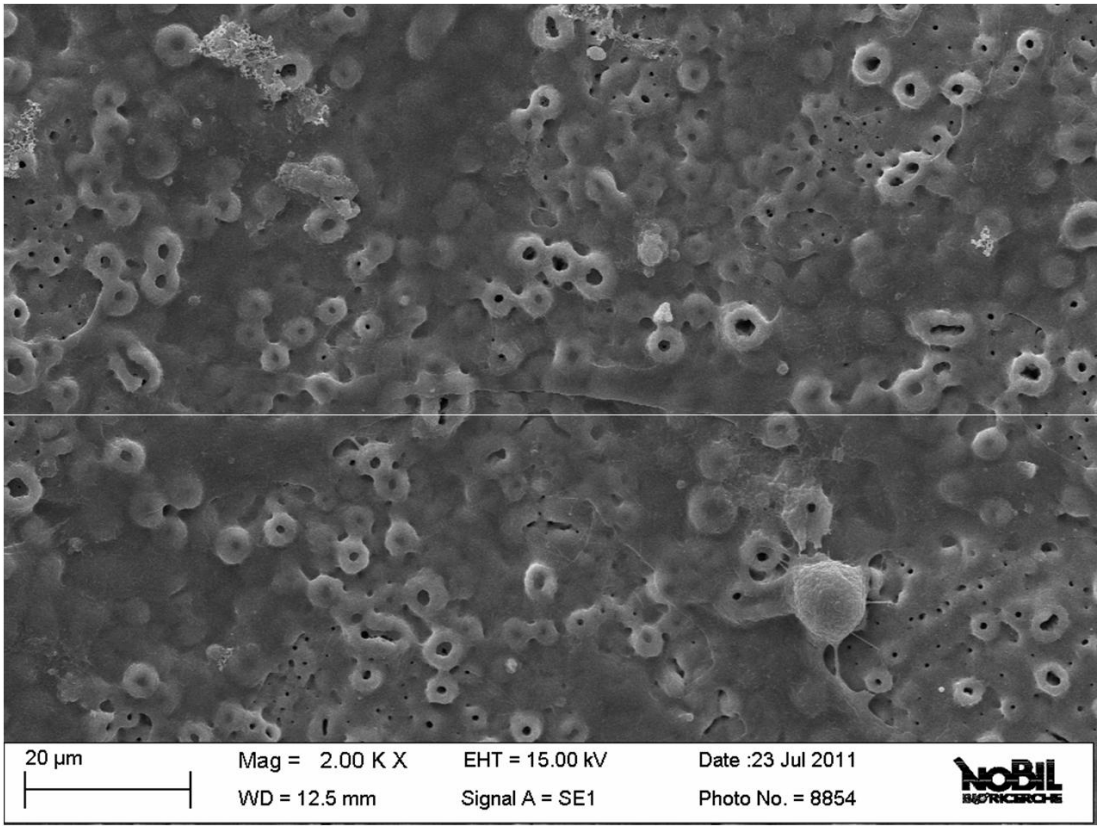

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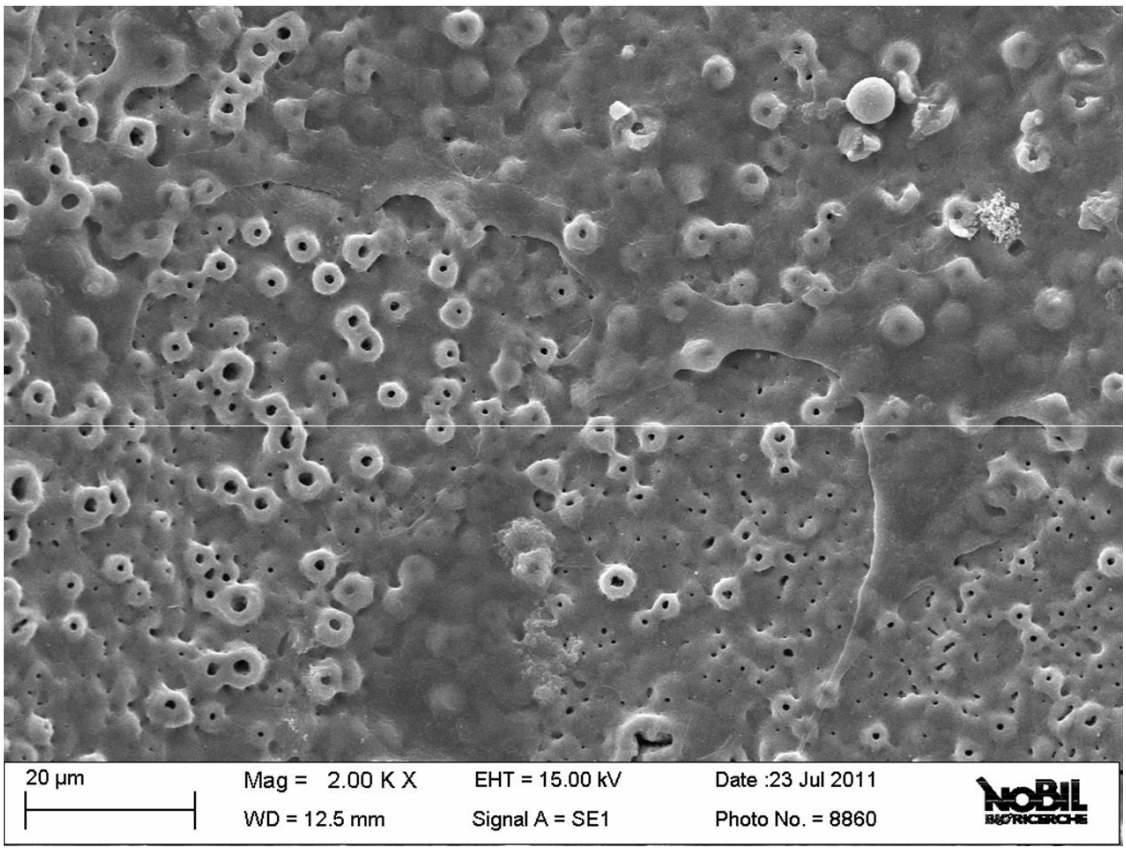

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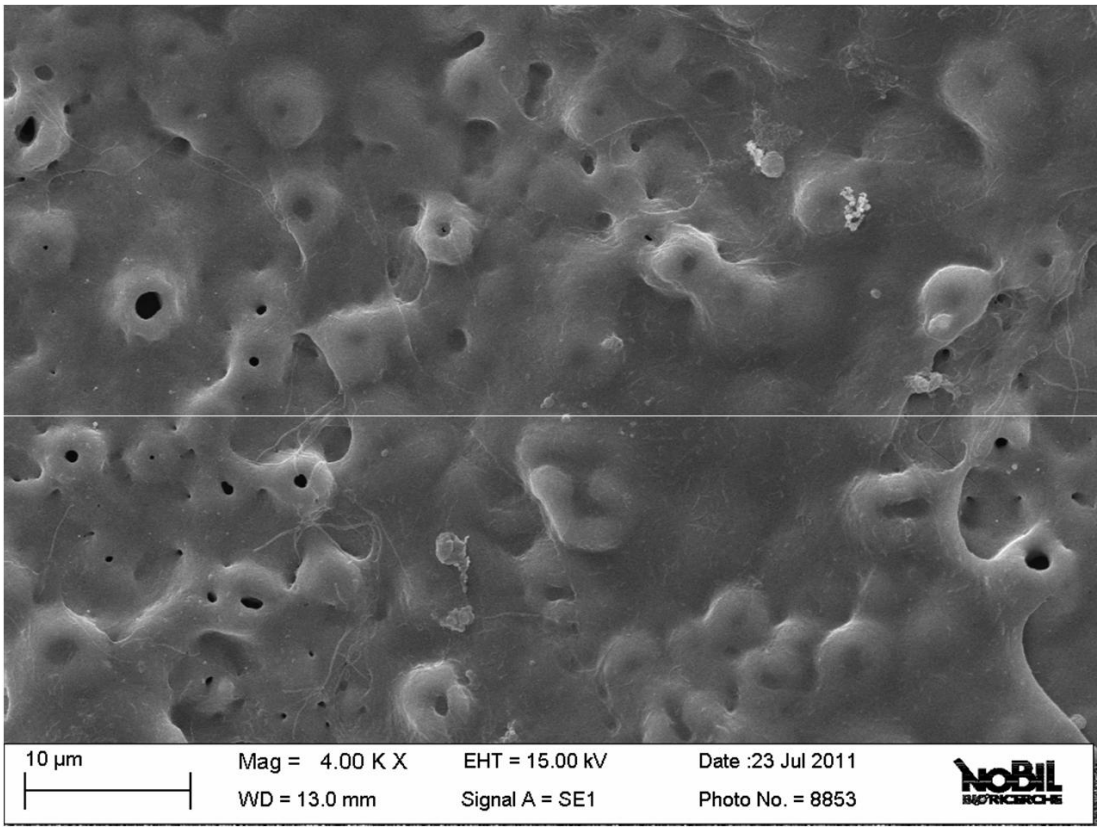

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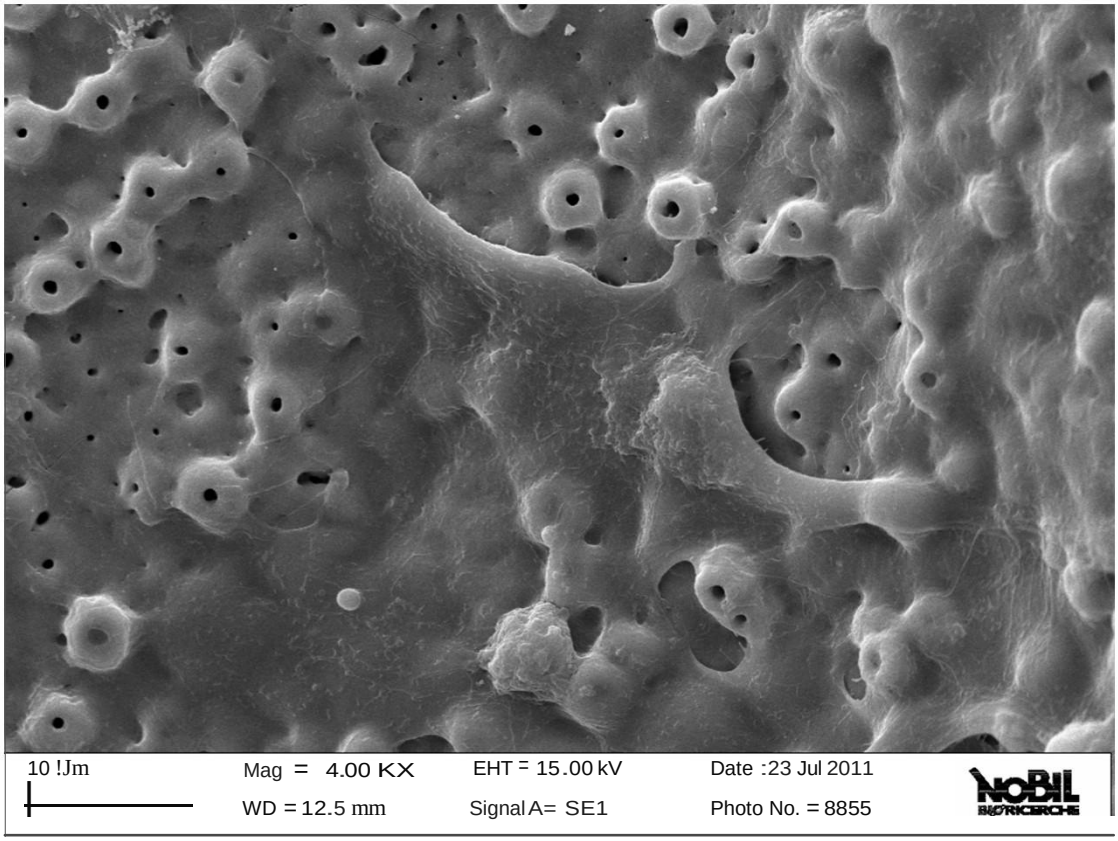
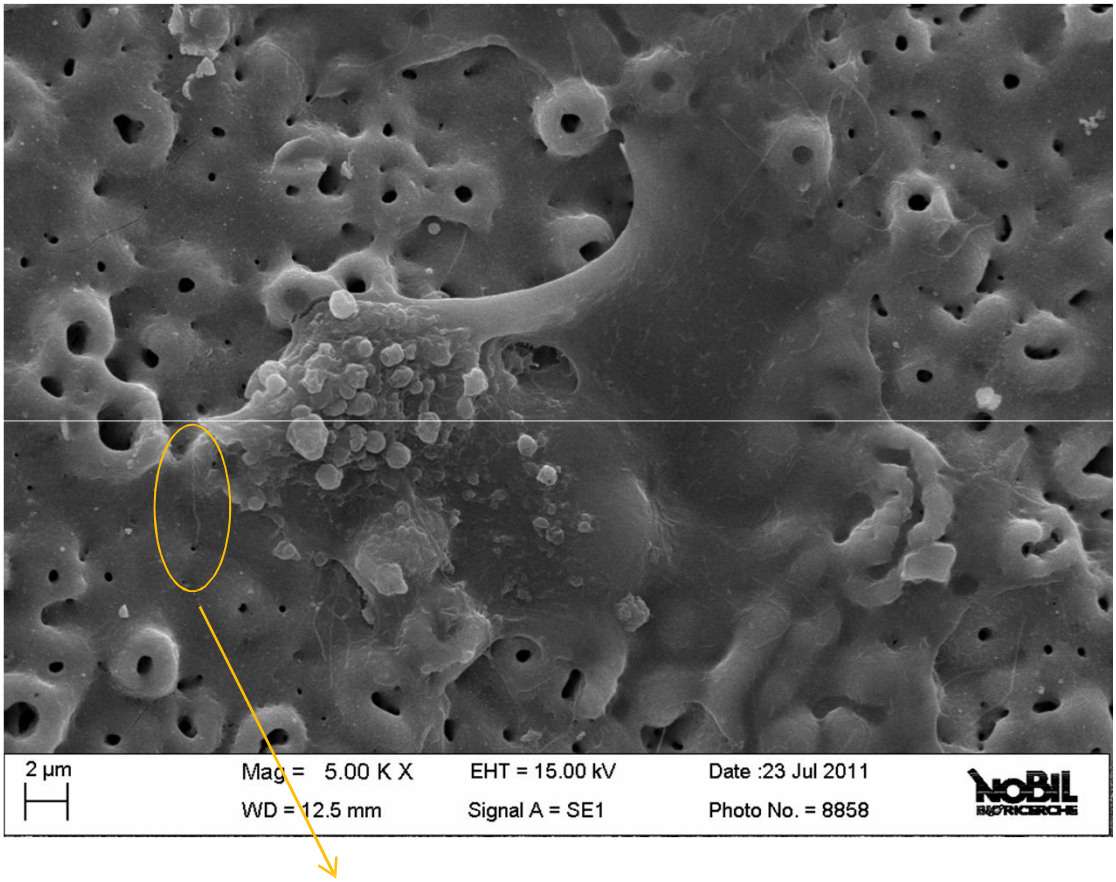


24 HOURS

Fig. 20



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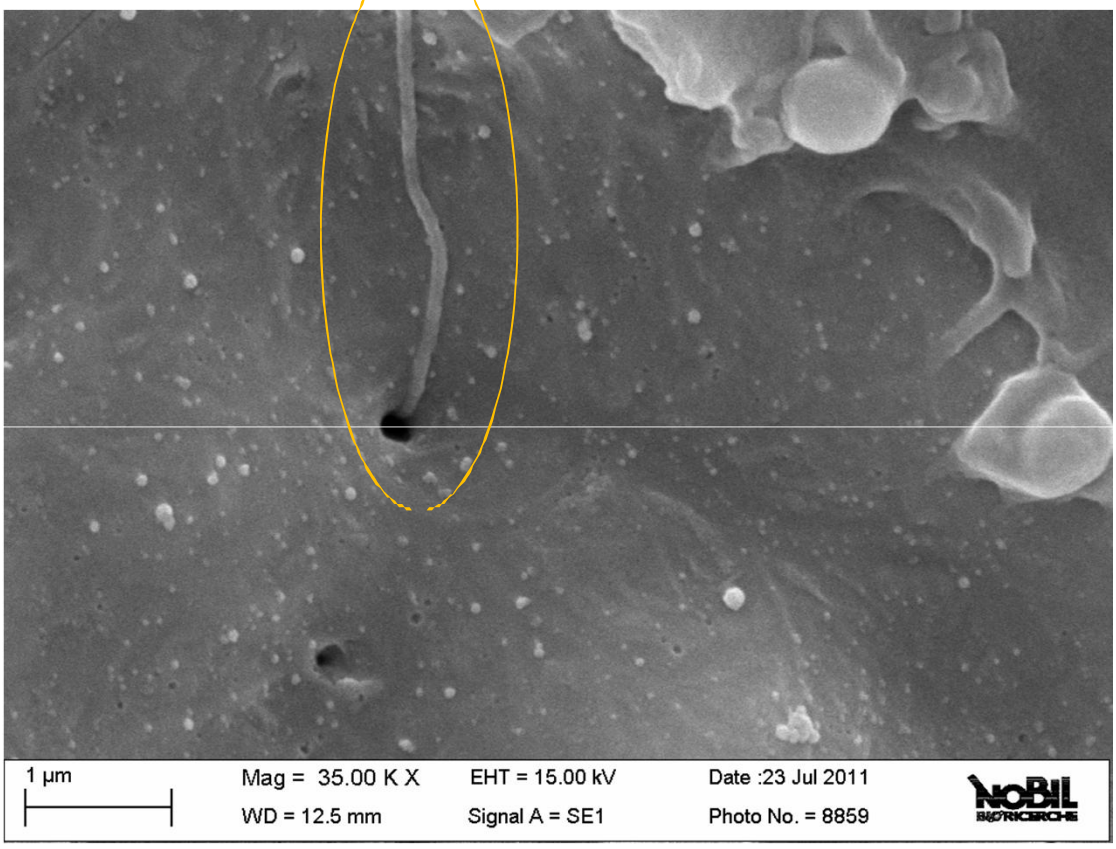
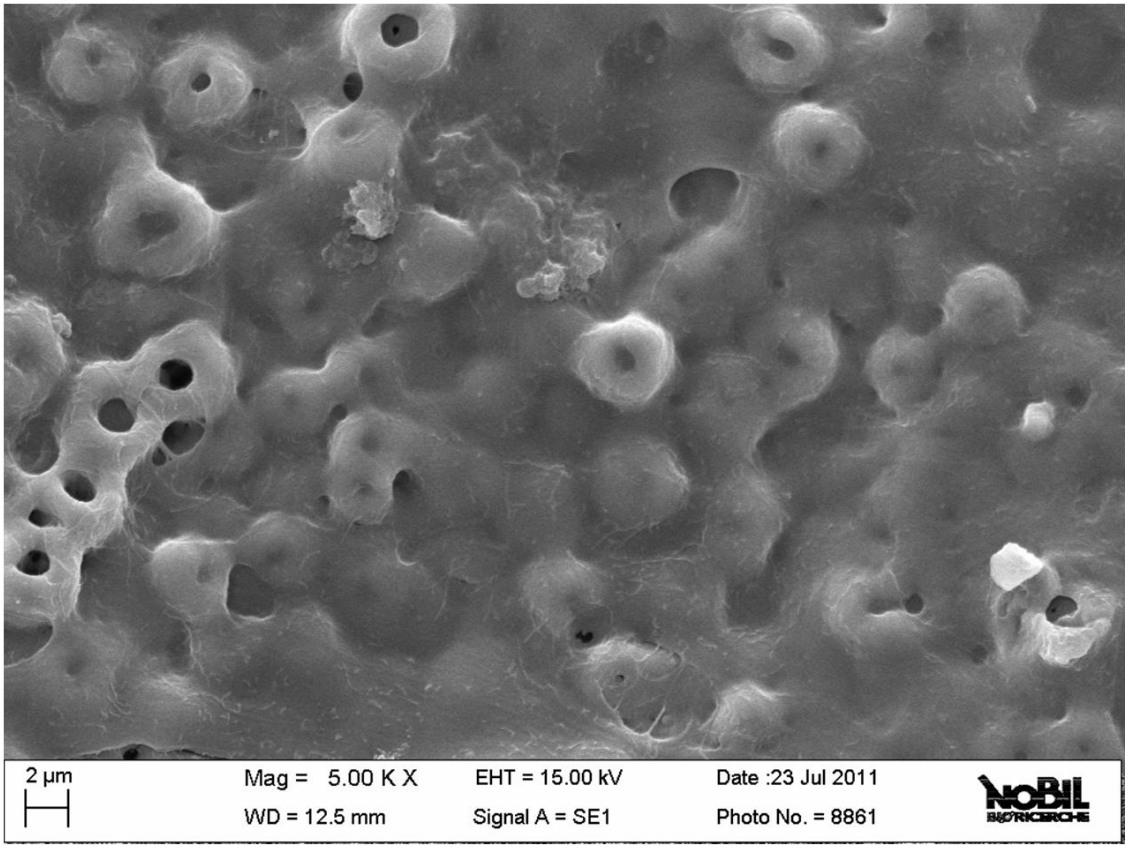


24 HOURS

Fig. 22



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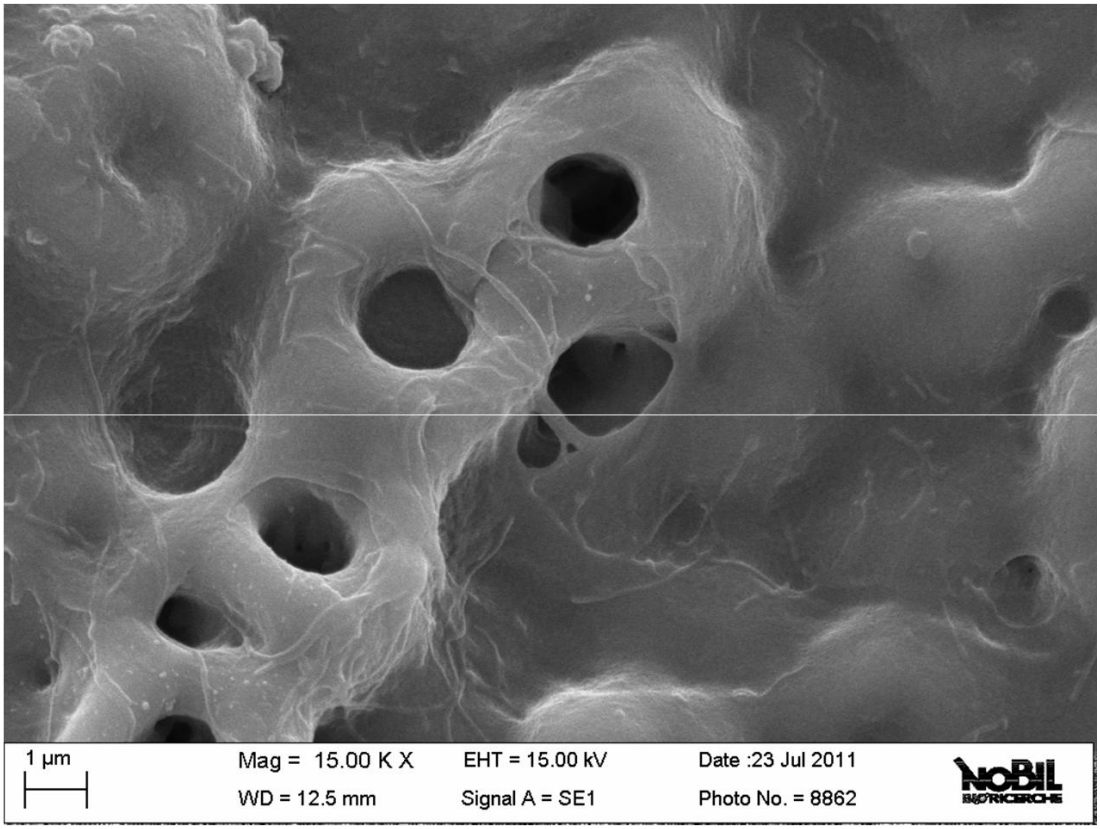
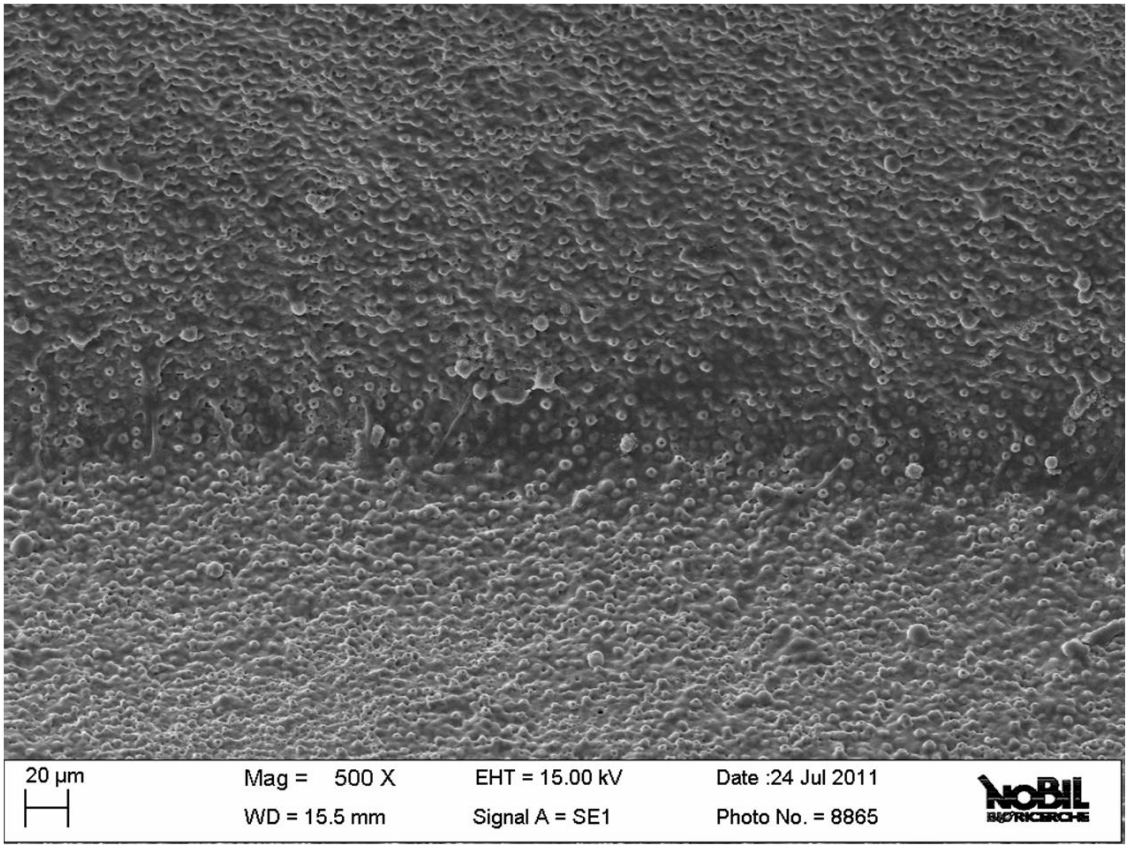


72 HOURS

Fig. 24



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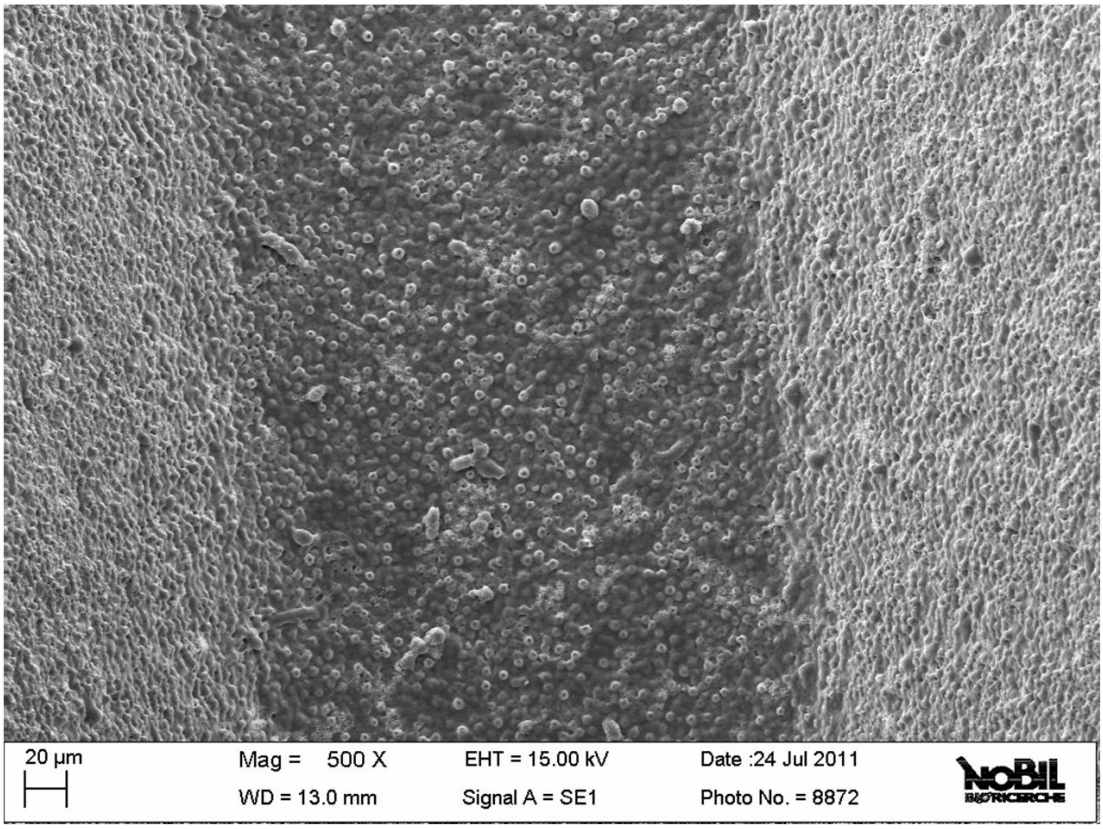

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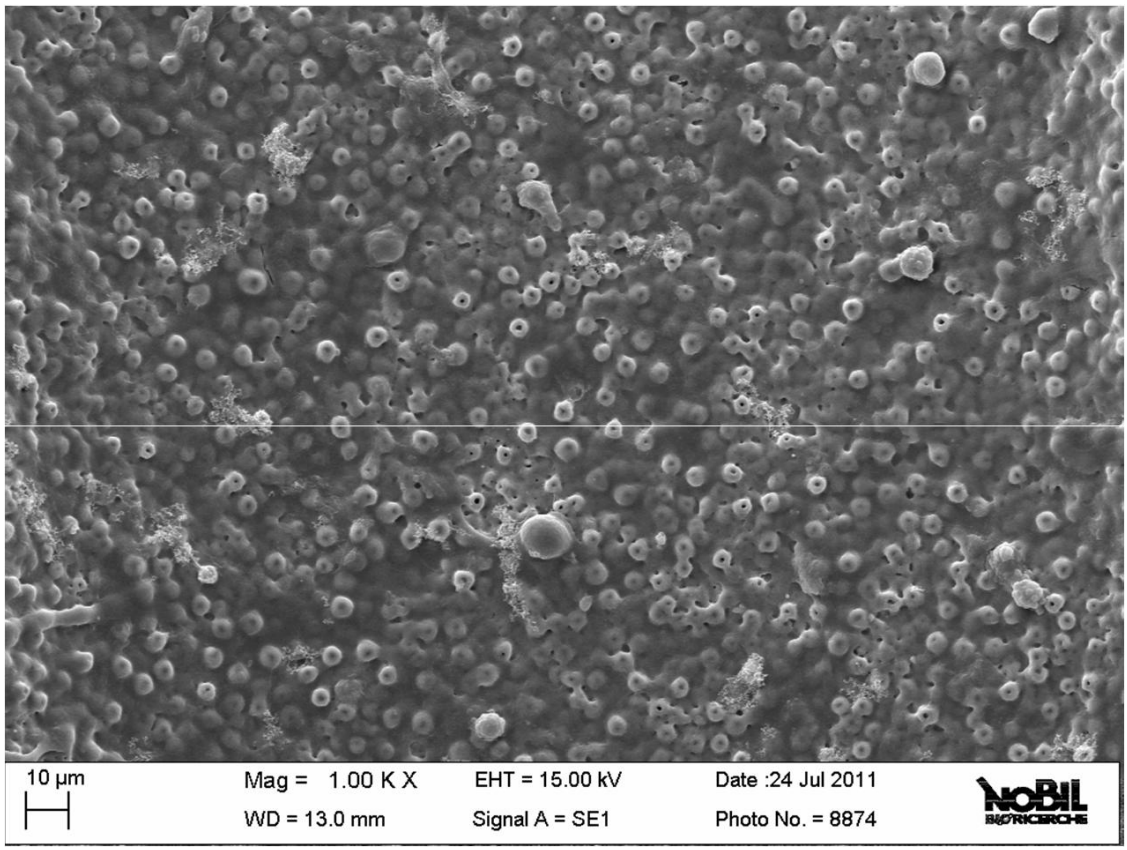

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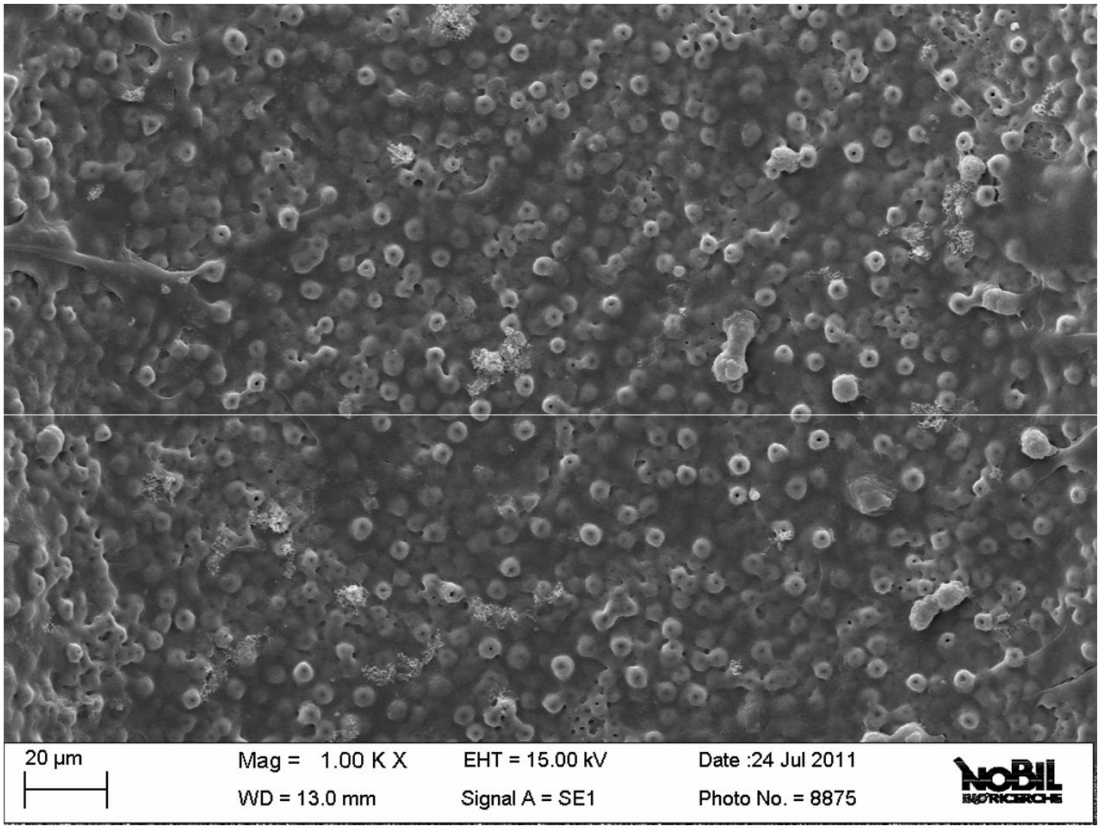

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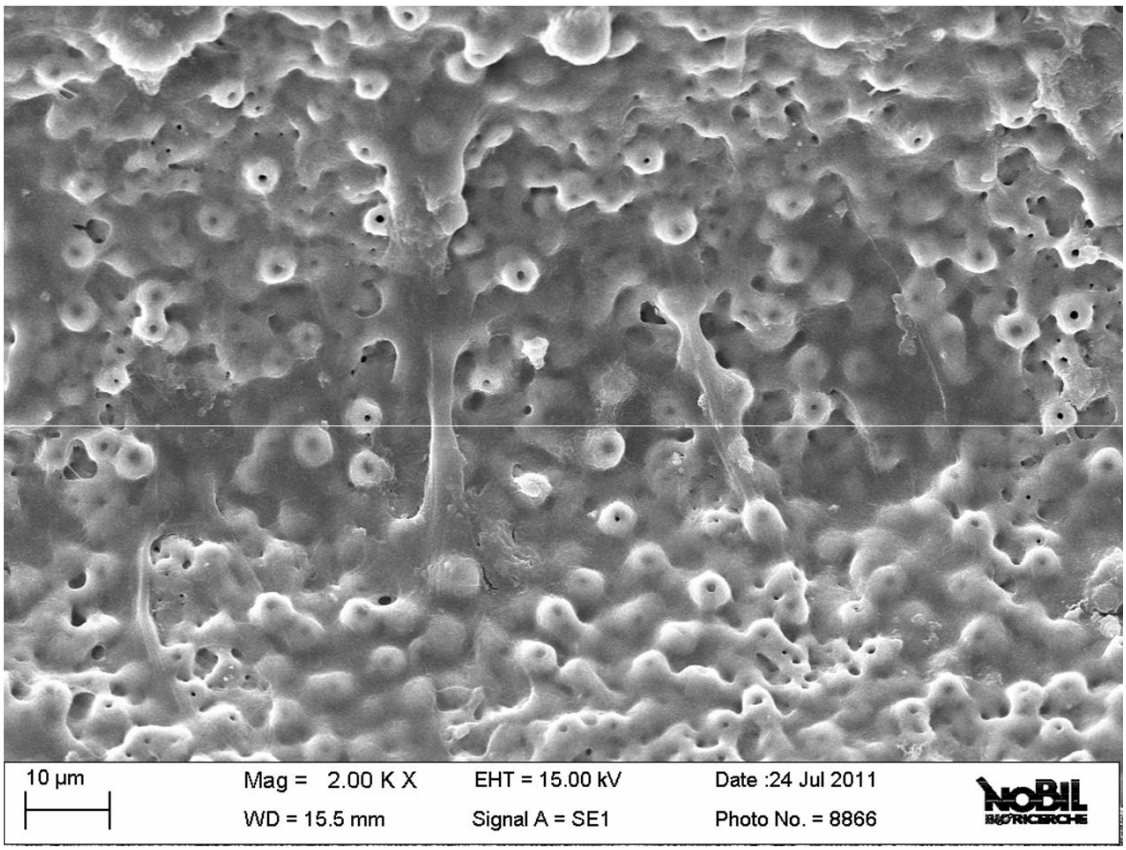

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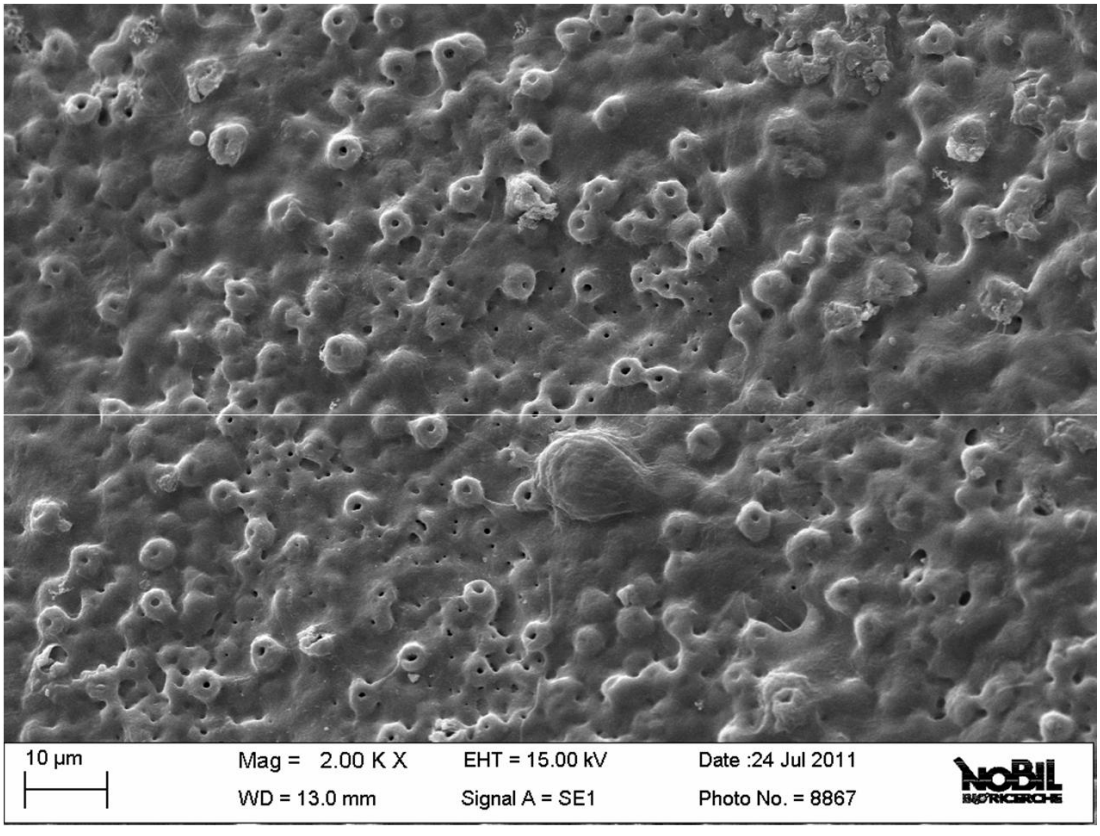

72 HOURS

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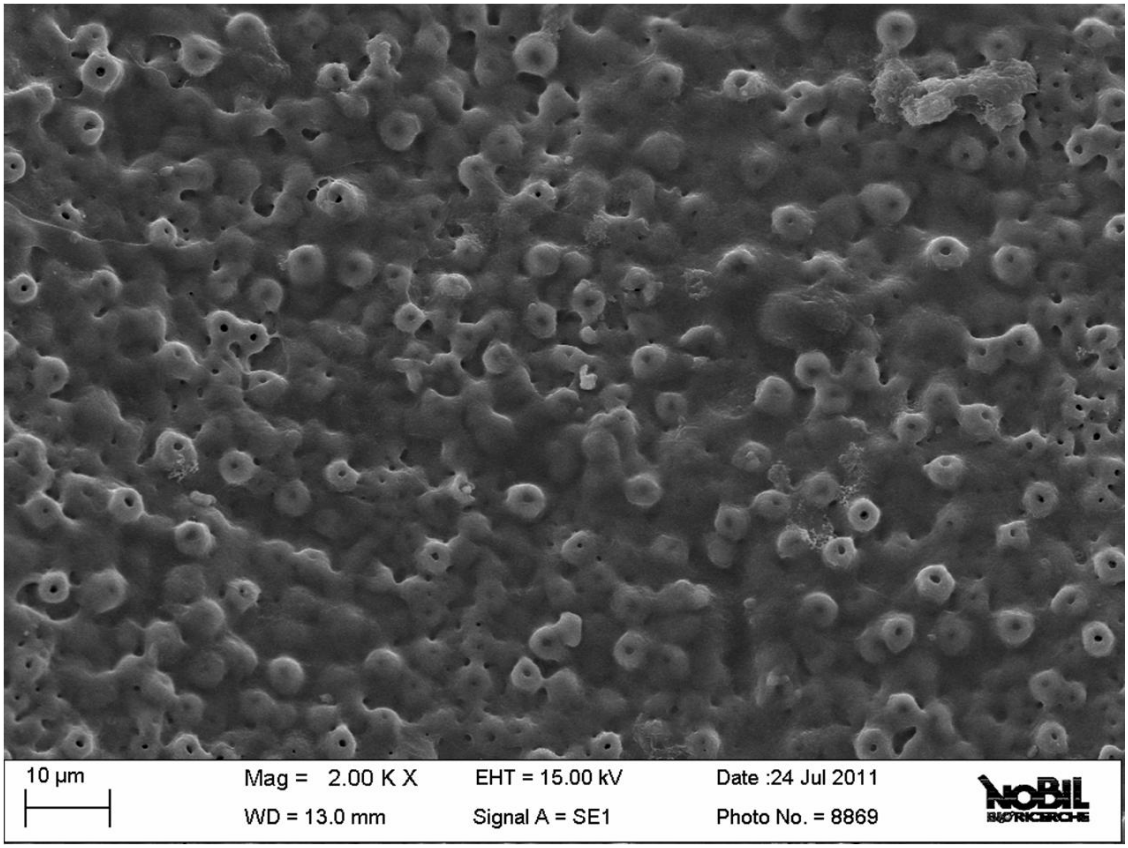

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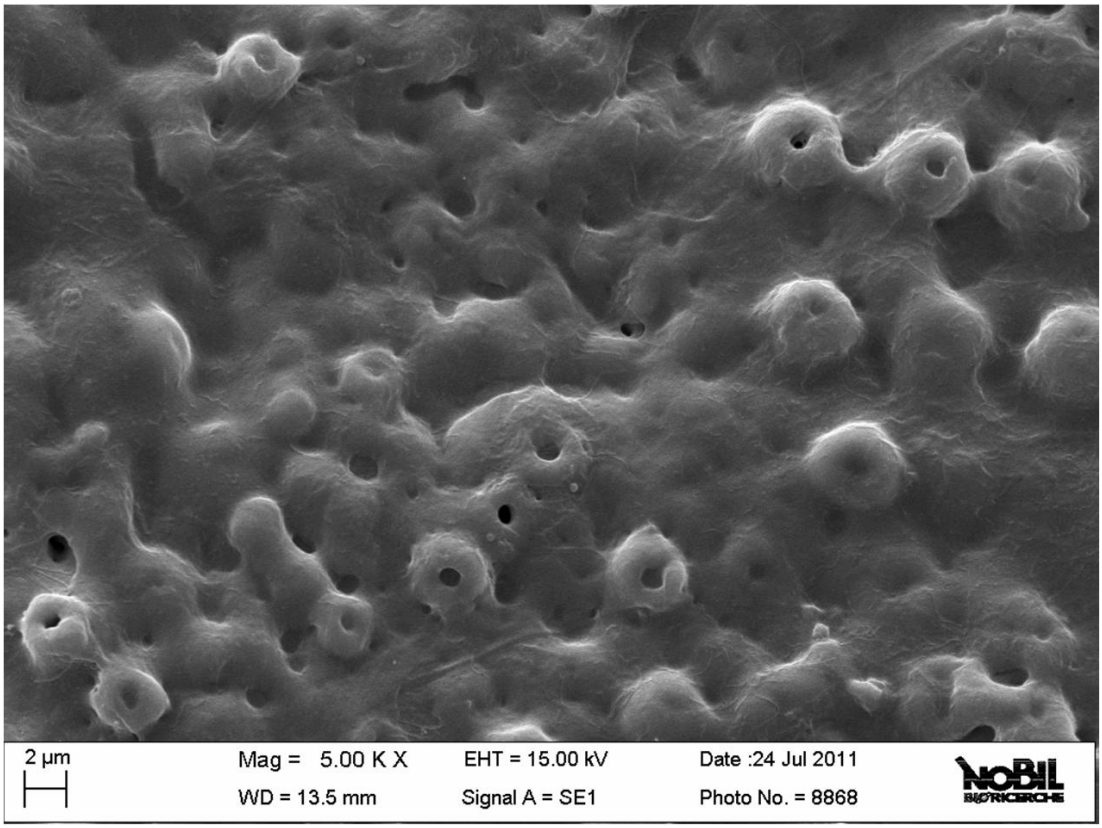

72 HOURS

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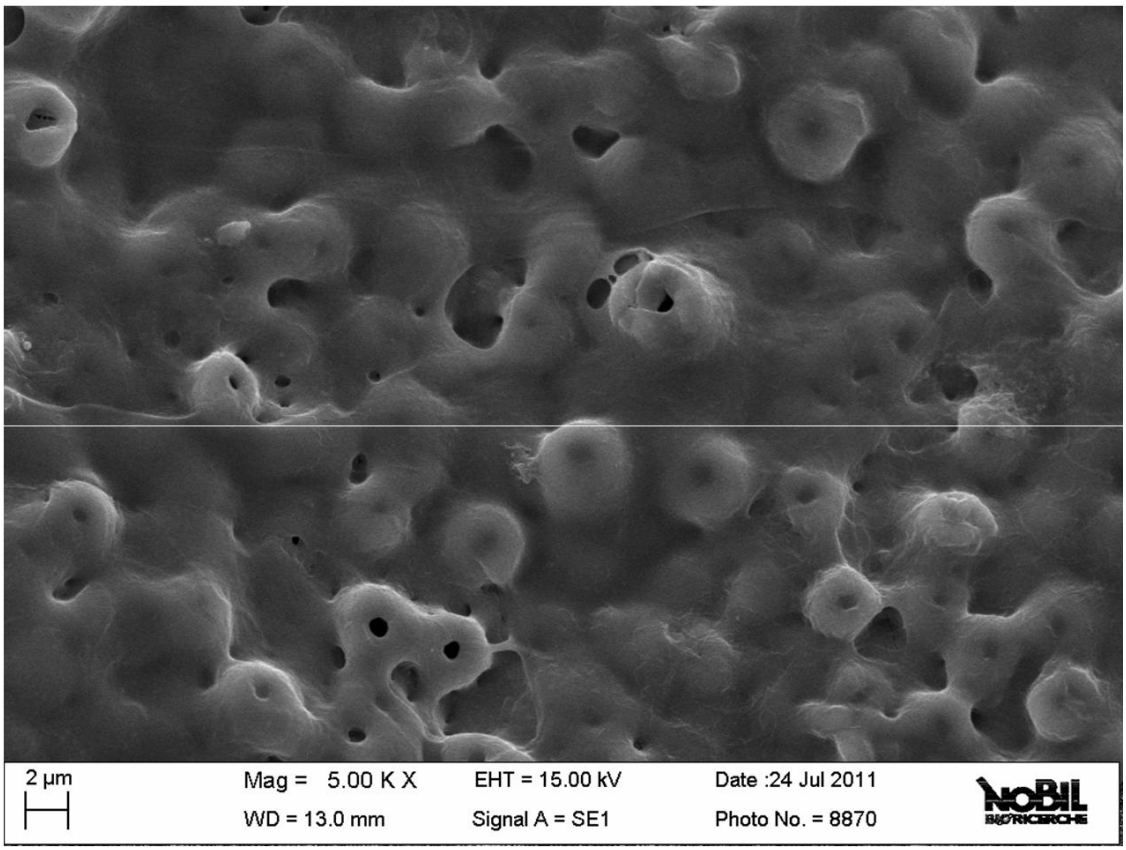

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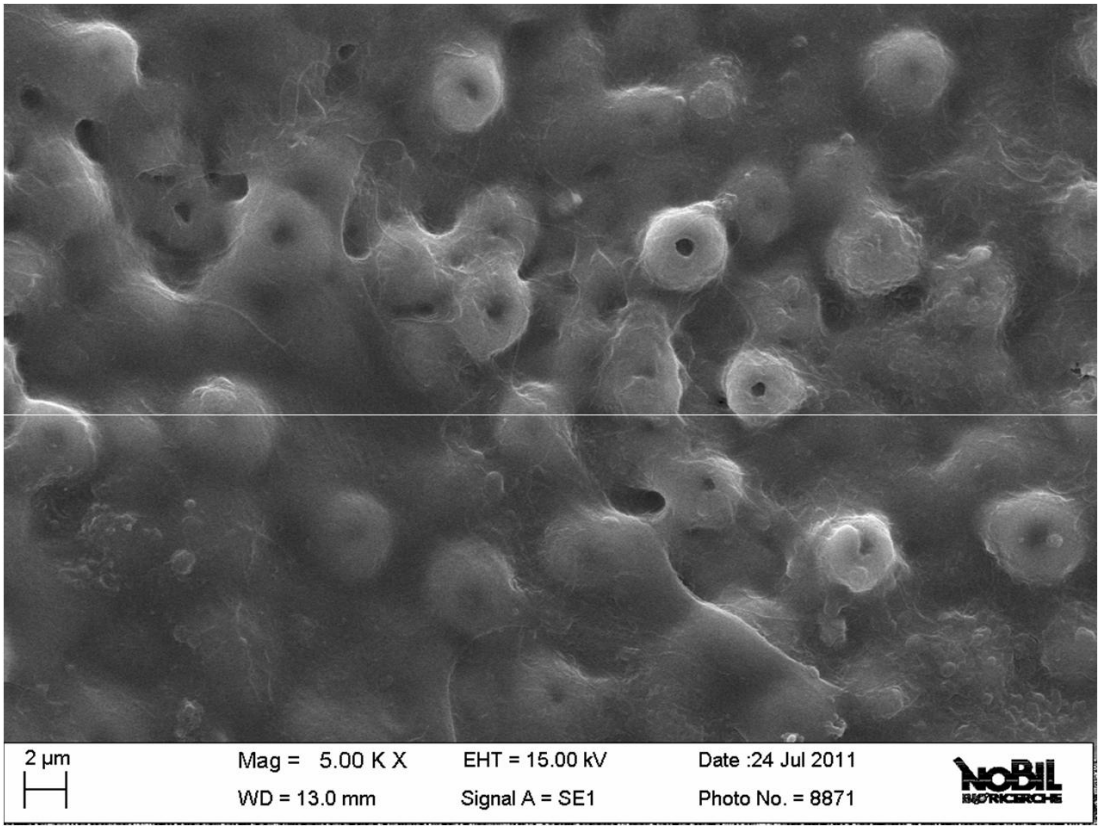

72 HOURS

Fig. 34