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Preliminary Study of Sterilizing Filtration Validation: Microscopy of DOT-IPEN-177 Filtration Membrane

V. L. T. Oliveira, L. J. Santos, L. C. Cavalcante,
L. A. P. Dias, C. F. Felgueiras and M. M. N. Matsuda.

contatoviniciuslimaoliveira@gmail.com, Nuclear and Energy Research Institute IPEN-CNEN
Av. Prof. Lineu Prestes, 2242 – Cidade Universitária
CEP 05508-000 – São Paulo – SP - Brazil

1. Introduction

Radiopharmaceuticals are defined as pharmaceutical preparations with diagnostic or therapeutic purposes, which, when ready for use, must meet the requirements applicable to the manufacture of sterile medicines, (except for radiopharmaceuticals for oral use). Sterilizing filtration is generally used as a method of choice to guarantee the sterility of radiopharmaceuticals that cannot be autoclaved, as is the case of DOT-IPEN-177 (*177 Lu-DOTATATE*), which is used in oncological treatment in neuroendocrine tumor therapy. The objective of this work is to carry out a preliminary study of the sterilizing filtration validation using scanning electron microscopy of the PVDF membrane after filtration of DOT-IPEN-177 radiopharmaceutical and *Pseudomonas paraeruginosa* microorganism.

2. Methodology

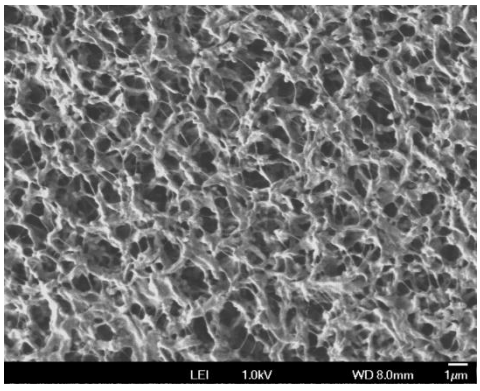
A scanning electron microscope (SEM-FEG) *Jeol* model JSM-6701F (Figure 1) was used to obtain the images of 0.22 μm *Millipore Millex-GV* filtration membrane (polyvinylidene fluoride - PVDF) (Figure 2): 1) as bought; 2) after filtration of DOT-IPEN-177; 3) after filtration of 5 mL of $4.5x10^8$ CFU (Colony Forming Units) of *Pseudomonas paraeruginosa NCTC 12924* (*BioMerieux Bioball*) suspension and 4) after filtration of DOT-IPEN-177 and *Pseudomonas paraeruginosa*. The residual activity of the filters used in the filtration of 177 Lu decayed and then the filters were opened to withdraw the membranes. In the experiments with *Pseudomonas paraeruginosa*, after passing the suspension through the filters, the microorganisms were fixed with ethanol PA and the membranes were dried. The membranes were covered with carbon to carry out the microscope analysis.

Figure 1: Scanning electron microscope.

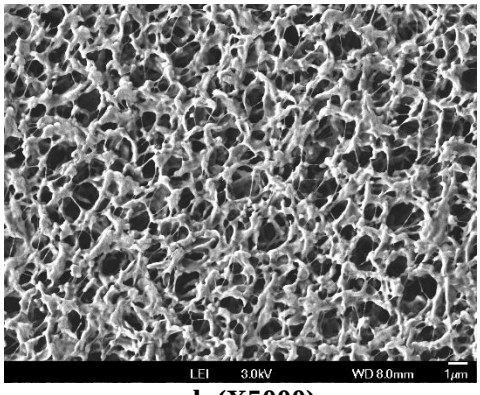
Figure 2: 0.22 μm *Millex-GV* filtration membrane *Millipore* (*PVDF*).

3. Results and Discussion

The validation of sterilizing filtration is defined by *Parenteral Drug Association (PDA)* document [1] which deals in detail with essential tests to prove the sterility of the product, as: integrity; membrane/product compatibility; study of adsorption, extractables, bacterial retention challenge and bacterial endotoxins. Visual knowledge of the membrane provides an additional way to confirm pore size up to 0.22 μm. In Figure 3, the images were taken with magnification of 5000, 8000 and 10000 times, and they confirm surface integrity and pore size as specified, and suitability for use in sterilizing filtration. After filtration with DOT-IPEN-177, deposition of some material on the membrane was observed in Figure 4. In Figure 5, *Pseudomonas paraeruginosa*, the surface of the membrane indicated retention of the microorganism of a similar size to *Brevundimonas diminuta* (Figure 6). In Figure 7 we observed deposition of the material and microorganism.



a (X5000)



b (X5000)

Figure 3: Microscopy of the membrane (a) and its back side (b) before filtration of DOT-IPEN-177

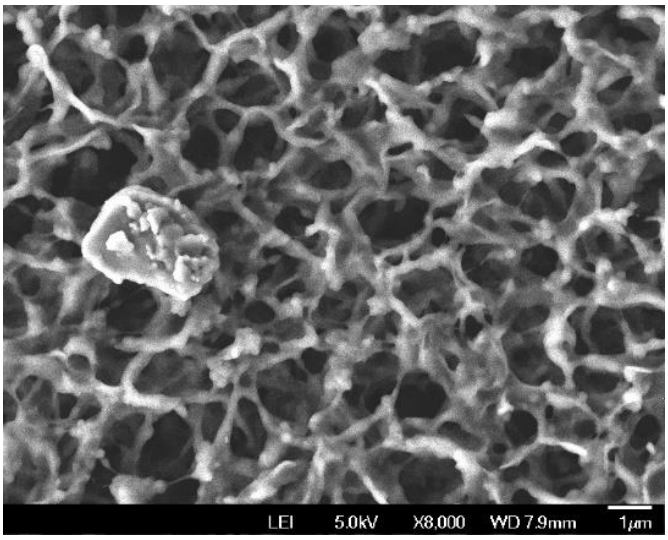


Figure 4: Membrane microscopy after DOT-IPEN-177 filtration.

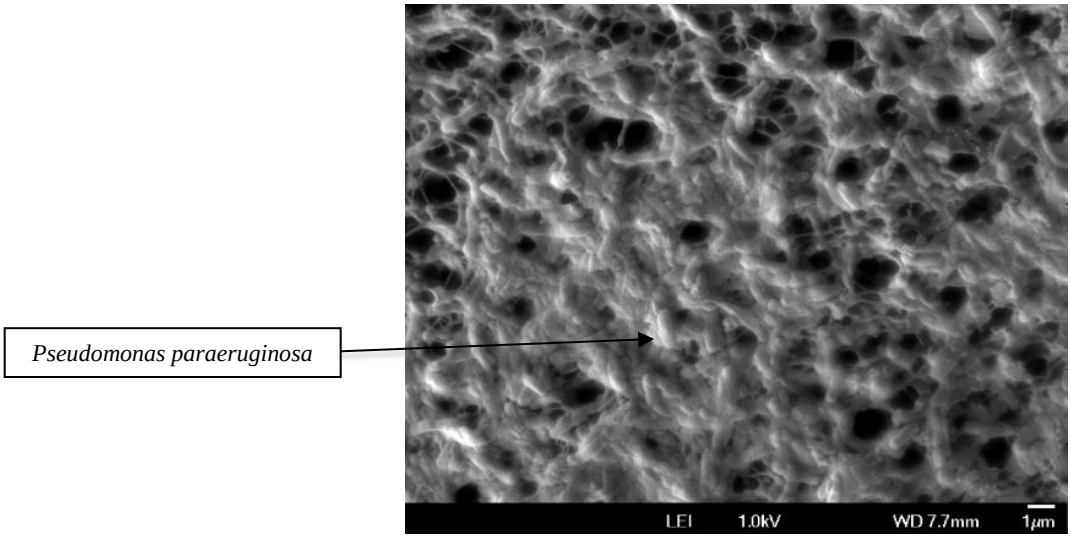


Figure 5: Microscopy image of the membrane after passage of *Pseudomonas paraeruginosa* with x5000 of magnification

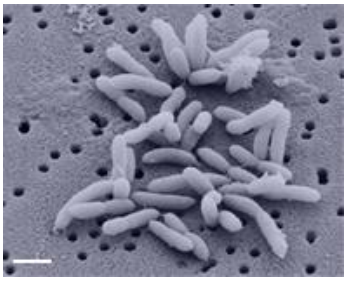


Figure 6: *Brevundimonas diminuta* in membrane of black polycarbonate with scale bar = 1 μm by *MicrobeWiki* (2013)

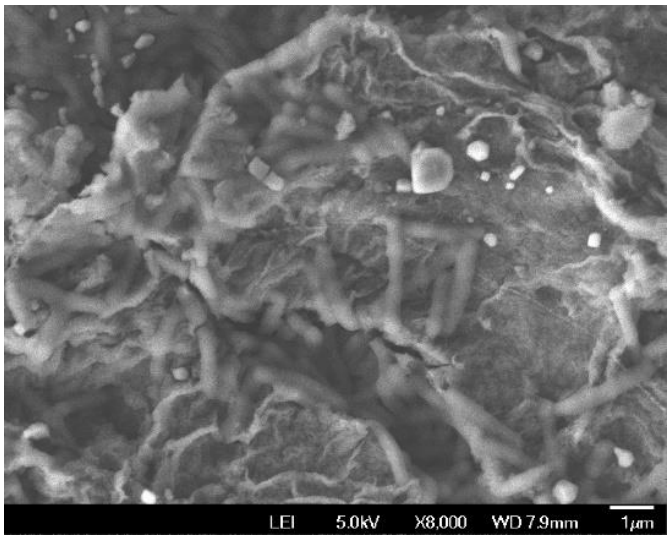


Figure 7: Membrane microscopy after passage of DOT-IPEN-177 and *Pseudomonas paraeruginosa* $4.5x10^8$ CFU.

4. Conclusions

SEM-FEG microscopy images after the passage of the radiopharmaceutical solution and *Pseudomonas paraaeruginosa* suspension through the 0.22 μm PVDF membranes indicate that sterilizing filtration of the radiopharmaceutical DOT-IPEN-177 is effective and the results of this preliminary study will be very useful to the sterilizing filtration validation.

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