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SCIENTIFIC ARTICLE

In vitro comparison of epidural bacteria filters permeability and screening scanning electron microscopy

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KEYWORDS
Bacteria filter;
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Abstract
Background and objectives: Epidural catheter bacteria filters are barriers in the patient-controlled analgesia/anaesthesia for preventing contamination at the epidural insertion site. The efficiency of these filters varies according to pore sizes and materials.
Method: The bacterial adhesion capability of the two filters was measured in vitro experiment. Adhesion capacities for standard *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) strains of the two different filters (Portex and Rusch) which have the same pore size were examined. Bacterial suspension of 0.5 Mc Farland was placed in the patient-controlled analgesia pump, was filtered at a speed of 5 mL/h. in continuous infusion for 48 h and accumulated in bottle. The two filters were compared with colony counts of bacteria in the filters and bottles. At the same time, the filters and adhered bacteria were monitored by scanning electron microscope.
Results: Electron microscopic examination of filters showed that the Portex filter had a granular and the Rusch filter fibrillary structure. Colony counting from the catheter and bottle showed that both of the filters have significant bacterial adhesion capability ($p < 0.001$). After the bacteria suspension infusion, colony countings showed that the Portex filter was more efficient ($p < 0.001$). There was not any difference between *S. aureus* and *P. aeruginosa* bacteria adhesion. In the SEM monitoring after the infusion, it was physically shown that the bacteria were adhered efficiently by both of the filters.

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Conclusion: The granular structured filter was found statistically and significantly more successful than the fibrial. Although the pore sizes of the filters were same – of which structural differences shown by SEM were the same – it would not be right to attribute the changes in the efficiencies to only structural differences. Using microbiological and physical proofs with regard to efficiency at the same time has been another important aspect of this experiment.
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PALAVRAS-CHAVE
Filtro antibacteriano;
Staphylococcus aureus;
Pseudomonas aeruginosa;
Microscópio eletrônico de varredura

Comparação *in vitro* da permeabilidade de filtros epidurais antibacterianos e triagem por microscopia eletrônica de varredura

Resumo
Justificativa e objetivos: Os filtros antibacterianos para cateter epidural são barreiras da analgesia/anestesia controlada pelo paciente para evitar a contaminação do local de inserção epidural. A eficácia desses filtros varia de acordo com o material e tamanho dos poros.
Método: A capacidade de aderência bacteriana dos dois filtros foi medida em experimento *in vitro*. Avaliamos a capacidade de aderência das cepas padrão de *Staphylococcus aureus* (ATCC 25923) e *Pseudomonas aeruginosa* (ATCC 27853) de dois filtros diferentes (Portex e Rusch), mas com poros do mesmo tamanho. Uma suspensão bacteriana grau 0,5 de McFarland foi colocada na bomba de analgesia controlada pelo paciente e filtrada a uma velocidade de 5 mL/h em infusão contínua por 48 h e acumulada em frasco. Os dois filtros foram comparados com contagens de colônias de bactérias nos filtros e frascos. Ao mesmo tempo, os filtros e as bactérias aderidas foram monitorados com microscópio eletrônico de varredura.
Resultados: O exame dos filtros por microscópico eletrônico mostrou que a estrutura do filtro Portex era granulada e a do filtro Rusch fibrilar. A contagem de colônias do cateter e do frasco mostrou que ambos os filtros possuíam uma capacidade de adesão bacteriana significativa ($p < 0{,}001$). Após a infusão da suspensão bacteriana, as contagens de colônias mostraram que o filtro Portex foi mais eficiente ($p < 0.001$). Não houve qualquer diferença entre as adesões de bactérias *S. aureus* e *P. aeruginosa*. Na monitoração por MEV após a infusão, ficou fisicamente evidente que as bactérias foram aderidas de modo eficaz por ambos os filtros.
Conclusão: O filtro com estrutura granular foi estatística e significativamente mais bem sucedido que o filtro com estrutura fibrilar. Embora o tamanho dos poros dos filtros fosse igual — as diferenças estruturais mostradas pelo MEV eram semelhantes —, não seria justo atribuir as alterações de eficiência apenas às diferenças estruturais. O uso ao mesmo tempo de provas microbiológicas e físicas para avaliar a eficácia foi outro aspecto importante deste experimento.
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Introduction

Bacteria filter

Local anaesthesia was implemented by James Leonard Corning in 1885 for the first time in a by injecting cocaine into the epidural sites of dogs.

Due to the fact that the local analgesia/anaesthesia decreases postoperative mortality and morbidity, its administration has increased. The increased analgesia/anaesthesia has brought up new problems. These primary problems are cardio toxicity, hypotension, motor block, and transposition of the catheter.

Local analgesia/anaesthesia infection has been seen at rates of 0.5–5.4%.[1,3,4] It has been suggested to pay attention to sterilisation to prevent infection as well as placing filters in the catheters.[1–4] For this purpose, new types of catheters and filters began to be used.[1] The filters were used for preventing particulates including glass, etc. from entering the epidural/spinal site and the development of infection. The intended purpose of using filters today is to prevent contamination during the administration of bolus at the epidural site.

In the practice of anaesthesia, there are many controversial issues such as whether the administration should be for a short-term or long-term, care services of the patients should be provided in the hospitals or at home, what type of catheter is the most suitable for patients, what types of analgesics or combinations should be administered to the patients, and what should be the change period of the bacteria filters. The importance of the bacteria filter has always been neglected among all these problems and has not been totally studied. Due to the fact that cost effective analyses in the healthcare field have gained importance in the 21st century, the efficiency and necessity of the bacteria filters are now being discussed.

When the literature was analysed, no experimental studies in the in vitro studies, including the patient-controlled

anaesthesia/analgesia (PCA), were encountered. The duration of stay of the epidural catheter, the condition of the catheter administration space, the characteristics of the material of which the catheter was made, the asepsis of the person who performers the administration and his/her following proper antisepsis procedures, his/her personal experiences and skills have been indicated to be important factors contributing to the risk of infection.[1,2,7,10] There is no marked difference between the infection and colonisation levels according to the catheter types administered today. The factors affecting the risk of infection are[7–13]:

A Patient factors
   1. Age of the patient (>65 years and <2 years)
   2. Existence of a chronic disease (malignancy, diabetes mellitus, chronic renal failure)
   3. Anatomic condition of the administration area (surgeries, trauma history, instrumentation history, chronic degenerative disease)
   4. Existence of another infection centre (Haematogenous spread)

B Factors of the administrators
   1. Not following the procedures of asepsis
   2. Not cleaning the administration area of the skin properly
   3. Traumatic administration (haematoma)

C Factors of catheter
   1. Non-existence of the bacteria filter
   2. Characteristics of the bacteria filter (Membrane surface space and the material of which it is made)

   There are two bacteria filters according to the structures of the filter materials;
   1. Polyvinyl chloride
   2. Cellulose acetate
   3. Duration of stay of the catheter

D Factors of the active microorganism
   1. Capacity of adhesion to the bacteria filter (making a bio film)
   2. Resistance to the sanitisers and antiseptics
   3. Taking part in the flora-colonisation

Purpose

Comparing the *Staphylococcus aureus* and *Pseudomonas aureginosa* bacteria adhesion capacities of the two different bacteria filters Portex® (Smiths-Medical, USA) and Rusch® (Melsungen, Germany) that are commonly used in daily practice with an in vitro testing apparatus and demonstrating the visual adhesion of the bacteria by the filter system with scanning electron microscopy.

Materials and methods

Bacteriological method and testing apparatus

The researcher who was going to prepare the testing apparatus wore sterilised clothes. The rubber top of the empty and sterile 1000 cc bottles was cleaned with a sterilised batticon twice. 18 G of *tuohy* syringe was placed into the top of the bottle. The catheter was put into the syringe and the syringe was removed. Catheter was located in the 9 cm distance from bottle. A filter was attached on the tip. In this study, Portex® (Smiths-Medical, USA) which has 0.2 μm of pore aperture and Rusch® (Melsungen, Germany) flat catheter filters were used. The differences between the filters are explained in Table 1.

Table 1 Summary of the differences between the filters.

Catheter	Surface space	Pore material	Filter material
Portex®	4.91 cm^2	Circular	Polyvinyl chloride
Rusch®	5.0 cm^2	String	Cellulose acetate

Experiment groups

GROUP 1: Portex filter 10×
S. aureus 0.5 Mfc (10^5 cfu/mL)-250 cc normal saline
GROUP 1 Control: Portex filter 10×
No bacteria-250 cc normal saline
GROUP 2: Portex filter 10×
P. aeruginosa 0.5 Mfc (10^5 cfu/mL)-250 cc normal saline
GROUP 2 Control: Portex filter 10×
No bacteria-250 cc normal saline
GROUP 3: Rusch filter 10×
S. aureus 0.5 Mfc (10^5 cfu/mL)-250 cc normal saline
GROUP 3 Control: Rusch filter 10×
No bacteria-250 cc normal saline
GROUP 4: Rusch filter 10×
P. aeruginosa 0.5 Mfc (10^5 cfu/mL)-250 cc normal saline
GROUP 4 Control: Rusch filter 10×
No bacteria-250 cc normal saline

The bacteria used in the study are clinical standard strains; *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853). Both bacterial suspensions were prepared in the sterilised saline serum with optic density of 0.5 Mc Farland (10^5 cfu/mL) and in the 250 mL of saline serum in a sterilised area. The tip of the pump set was attached to the filter. The suspension which was filtered for 48 h in a continuous infusion of 5 mL/h by using the PCA (Patient Controlled Analgesia) equipment was accumulated in the syringe.

Bacterial isolation and identification were done from the pumping site in aerobic conditions of 37 °C after 16–24 h incubation. The number of the colony was examined to check if there was any decrease. After infusion, bacteria filters were cleaned with sterile normal saline and bacterial isolation and identification was also performed for cleaning solution. Bacterial adheration to filters and proportion of the bacteria held by filters were compared.

Electron microscopic screening

In the electron microscopic screening, SEM (scanning electron microscope) JEOL-JSM-6060 model was used. The examples were dried with a method of critical-point drying and they were covered by Gold-Palladium and 'Sputter Coater Poloran' method. Images were taken in the 15–20 kV range and photographic enlargements of 30×, 250×, 500×, 1000×, 2500× and 5000× were taken and saved.

In this study, the bacteria adhesion capacities were measured by filtering the bacterial suspension for a period of time. Moreover, since a physical proof was also required for

the bacteria adhered in the filters, SEM screening, which is interpreted as the gold standard, was done.

Statistical analysis

After the two different bacteria suspensions were filtered, the bacteria adhesion levels of the filters were compared by means of Man Whitney *U*, which is a nonparametric test by using SPSS 10.0 and the values under $p < 0.05$ were accepted as statistically significant.

Results

The fibrillary (Rusch-Fig. 1) and granular (Portex-Fig. 2) structures of the bacteria filters were monitored by SEM before the bacterial infusion. After the infusion of suspensions, the adhesions of the *S. aureus* (Figs. 3 and 4) and *P. aeruginosa* (Figs. 5 and 6) were monitored visually by SEM.

As a result of the comparison of the colony counts from the catheter and bottle, it was confirmed that both of the epidural filters demonstrated bacteria adhesion capacity at a significant level ($p < 0.001$) (Tables 2 and 3).

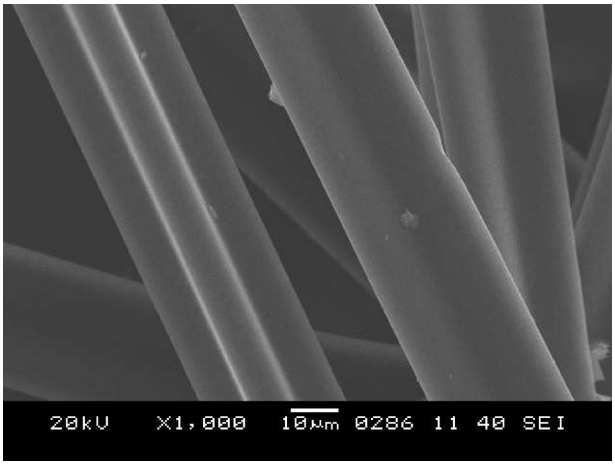


Figure 1 SEM-Rusch Flat Filter; Fibrillary structure 1000× magnification-before bacterial infusion.

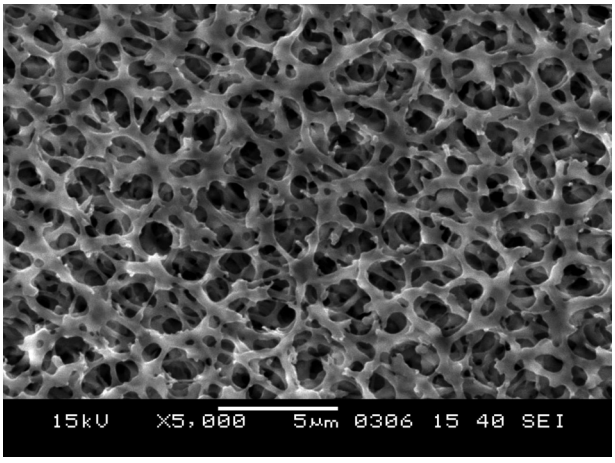


Figure 2 SEM-Portex Flat Filter; Granular structure 5000× magnification, before bacterial infusion.

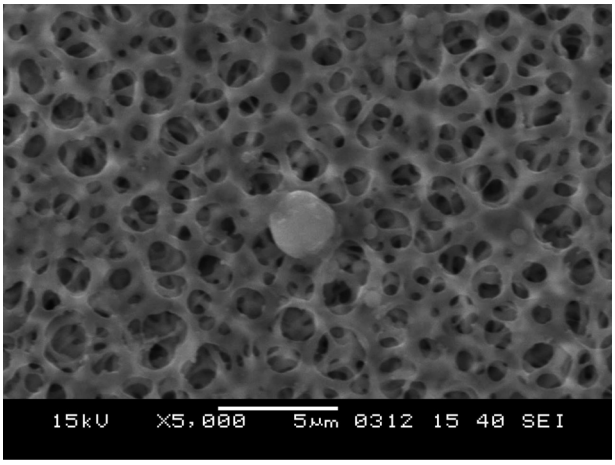


Figure 3 SEM-Portex Flat Filter, *Staphylococcus aureus* 5000× magnification, after bacterial infusion.

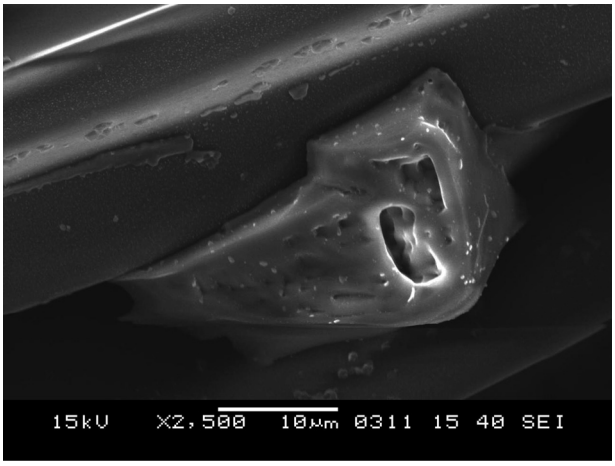


Figure 4 SEM-Rusch Flat Filter, *S. aureus* 2500× magnification, after bacterial infusion.

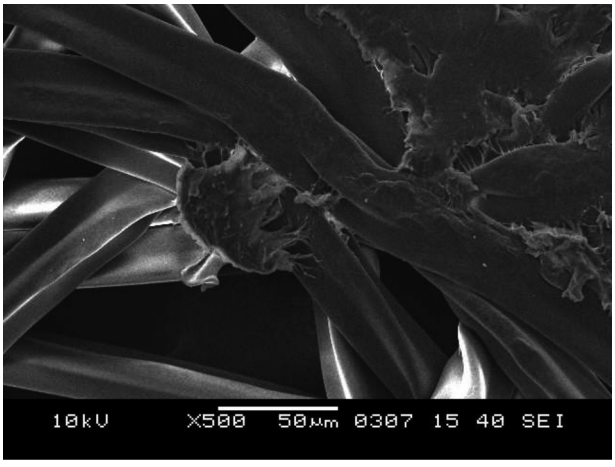


Figure 5 SEM-Rusch Flat Filter, *Pseudomonas aeruginosa* 500× magnification, after bacterial infusion.

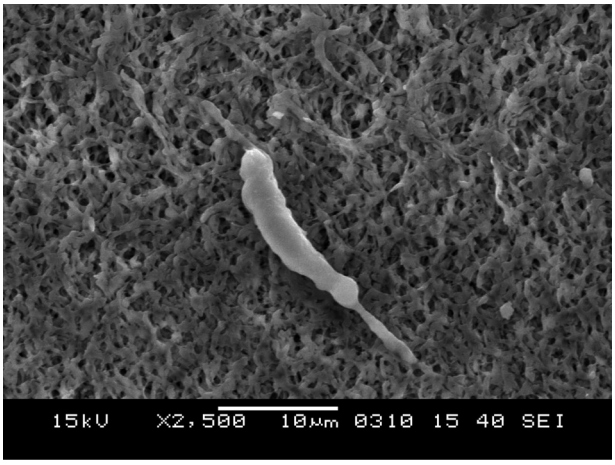


Figure 6 SEM-Portex Flat Filter, *P. aeruginosa* 2500× magnification, after bacterial infusion.

When the two different catheter filters were compared with each other, there was not any significant difference (Table 4).

When the colony counts from the bottles were compared after the bacterial suspension infusion, it was shown that the Portex® filter adheres to much more bacteria ($p < 0.001$) and it is more efficient.

No difference was observed in the filter adhesion of the *S. aureus* and *P. aeruginosa* bacteria.

Discussion

Two different bacteria filters which are used widely in the market were compared according to their efficiency in an in vitro study. A similar study in the literature was examined by De Cicco et al. for long-term pain control in epidural administrations about the efficiency of bacteria filters as in vivo and the efficiency was found to be acceptable.[2] The main subject of the discussion on this topic started with questioning whether there is a necessity to use bacteria filters in short-term epidural catheter administrations.[14] In fact, the first interpretation in the literature was brought up by Abouleish and Amortegui in 1977 with the claims whether bacteria filters are necessary in epidural anaesthesia which is especially used for labour.[15] In the following years, the fact that there were various numbers in the frequency of complications such as bacterial meningitis and epidural abscess in long-term epidural catheterisation caused a number of researchers and clinicians to be unclear about this issue.[16–21]

Table 2 Comparison of the colony counting from the catheter and bottle after infusion.

	$X \pm SD$	Significance
Rusch-catheter	190,000.0 ± 284,604.9894	MWU = 9000
Rusch-bottle	22,600.0 ± 40,958.5156	$p < 0.001$

Table 3 Comparison of the colony counting from the catheter and bottle after infusion.

	$X \pm SD$	Significance
Portex-catheter	91,000.0 ± 28,460.4889	MWU = .500
Portex-bottle	1243.0 ± 3101.7882	$p < 0.001$

Table 4 Comparison of bacterial colony counting of Portex and Rusch filters.

	$X \pm SD$	Significance
Rusch	190,000.0 ± 284,604.9894	MWU = 40.500
Portex	91,000.0 ± 28,460.4889	$p > 0.05$

This study is also crucial for having a rough idea whether bacteria filters should be administered in short-term (48 h) labour analgesia practically and scientifically. When we evaluated two different bacteria filters independently, we found them in vitro efficient. Both of the filters adhered bacteria efficiently and sufficiently (Tables 2 and 3). However, we need to add that although there is not significant difference between them statistically, the number of colony that Portex filter adhere is ten times more than the number of colony that the Rusch filter adhere (Table 4). In the enumeration (symbolises the epidural site in vivo) of colonies that was experimented in the bottles in both testing apparatus, the permeability of Portex bacteria filter was observed to be less (Table 3). According to our study, the efficiency of the Portex filter is higher.

The bacteria used in our study are standard clinical strains and they are the most frequent bacteria among Gram positive and Gram negative bacteria; *S. aureus* and *P. aeruginosa* which we come across as hospital infections. In the pilot study, it was determined that the adhesion capacity of the filters in different concentrations did not change. For this reason, the experimental group in which the bacteria used in intense concentration (1 McFarland) was removed from the study.

With the purpose of demonstrating the bacteria adhesion physically, SEM screening was taken in the study. In the screening which was taken before the bacteria infusion, the bacteria filters were found to be significantly different in structures. It was monitored that Rusch filters had fibrillary structure (Fig. 1) and Portex filters had a more concentrated granular structure (Fig. 2). The fact that their physical structures were demonstrated to be different explains the statistically significant difference in the bacteriological analyses. There are no other studies in literature where the bacteriological analysis supported the SEM images. The study has also been beneficial with regard to the fact that SEM images and visual demonstration of the bacteria adhesion by filters were demonstrated at the same time (Figs. 3–6). The fact that the bacteria adhesion was observed physically also shows the success of the testing apparatus.

The most important problem we encountered during the SEM screening was the fact that some images could not be taken clearly since the bacteria were sensitive to the >10 kV electron flow.

That there was no contamination in the synchronised control group of each infusion in the testing apparatus shows that we worked in sterilised conditions. This was confirmed by a random SEM screening.

Cost effective analyses of the materials that are used in healthcare field today gain importance day by day. De Cicco

evaluated the contamination risk in long-term bacteria filter usage and found that they were open to the contamination risk at higher levels than other materials in the in vitro study he performed.[2] In the retrospective studies that Low and his colleagues performed in the following years, they showed that the infection levels were low in epidural analgesia (for labour-short-term) in wide patient series.[4] For this reason, some epidural analgesia catheter sets are put on the market without bacteria filters. In our in vitro study, it was indicated that the bacteria in high density were adhered by the filters. In practice, however, this number of bacteria at this density cannot be used in long-term analgesic administrations. This study as a model opens the necessity of the bacteria filters in short-term epidural catheterisation when cost effective analysis prioritised up for discussion.

Especially in the long-term epidural analgesia administrations on cancer patients, it is a must to use the bacteria filters due to the fact that the duration is too long and promoter associated disease of the patient. Wallace and Du Pen who worked in this field a lot had the same results from his research.[1,8] Another exception of this case is short- or long-term epidural administrations that will be performed in childhood. In their study, Wood and his colleagues concluded that a bacteria filter should necessarily be used in all epidural administration which will be performed on children.[7] We evaluated the efficiency of bacteria filters in two bacteria basis with an in vitro testing apparatus and compared their differences. According to our hypothesis, granular filter was supposed to be statistically and significantly more successful in adhesion of dense bacteria suspension than the ones with fibria and we came to this conclusion. This experiment is a first microbiological and physical proof regarding the efficiency of the bacteria filters and also the first study in the literature that it will be used.

Conflicts of interest

The authors declare no conflicts of interest.

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