Reduction of biofilm formation with trisodium citrate in haemodialysis catheters: a randomized controlled trial

Jacob W. Bosma, Carl E.H. Siegert, Paul G.H. Peerbooms and Marcel C. Weijmer

Department of Nephrology and Dialysis, Department of Medical Microbiology and Infection Control, Saint Lucas Andreas Hospital, Amsterdam, The Netherlands

Correspondence and offprint requests to: Jacob W. Bosma; E-mail: j.w.bosma@student.vu.nl

Abstract

Background. Formation of an intraluminal microbial biofilm is noted to play a significant role in the development of catheter-related infections (CRIs). Recently, it has been demonstrated that trisodium citrate (TSC) has superior antimicrobial effects over heparin for catheter locking. In this randomized controlled trial, we compared the influence of catheter locking with heparin and TSC on the in vivo intraluminal biofilm formation in haemodialysis catheters.

Methods. Six patients were studied from the time of catheter insertion for haemodialysis treatment. They were randomly assigned to TSC 30% or heparin 5000 U/ml for catheter locking for the duration of 1 month. After elective guidewire exchange of the catheter, the locking solution was also changed. After removal, catheters were dissected in three segments and examined by standardized scanning electron microscopy (SEM) to assess quantitative biofilm formation. Furthermore, standardized cultures of all segments were performed to identify any microorganisms.

Results. In catheters filled with TSC, the average coverage by biofilm was 16% versus 63% in the heparin group (P < 0.001). A total of eight subsegments were associated with local catheter infection in the patients who were random-
itized to heparin locking versus three subsegments who were assigned to TSC ($P < 0.05$).

**Conclusions.** Our study demonstrates that using TSC 30% for catheter locking reduces the formation of microbial biofilm in haemodialysis catheters and culture-positive colonization. It is likely that this is the explanation for the observed prevention of CRIs by TSC locking.

**Keywords:** biofilm; catheter-related infections; catheter locking solutions; dialysis; electron microscopy; vascular access

---

**Introduction**

Catheter-related bacteraemia is a major cause of morbidity and mortality in haemodialysis (HD) patients. Recently, advances have been made in the prevention of catheter-related bloodstream infections (CRBSIs). Especially, the use of catheter lock solutions, placed in the catheter lumen after a dialysis session to maintain catheter patency, has been recognized as an effective manner in preventing CRBSIs, probably by reducing biofilm formation. [1,2]

Biofilms consist of dense aggregates of surface-adherent microorganisms embedded in a ‘slimy’ polysaccharide matrix. When cocooned within the fabric of the biofilm, microbes are protected from antimicrobial agents and host immune responses. By this mechanism, the presence of bacterial biofilms on catheter surfaces can serve as a nidus for infection and bacteraemia. In HD catheters, microorganisms can adhere to the surface of a catheter. Especially, contamination of the catheter hub, subsequent microbial colonization of the catheters and formation of a predominately intraluminal biofilm are thought to be major risk factors in the pathogenesis of catheter-related infections. [1,3–7]

Solutions containing trisodium citrate (TSC) solutions have been shown to have antimicrobial effects in vitro [8]. The antimicrobial activity was dose-dependent with the highest efficacy for TSC 30%. Presumably, this is because the construction and maintenance of a biofilm depends on cations, mainly Mg$^{2+}$ and Ca$^{2+}$ [9,10]. TSC is a strong chelator of both Mg$^{2+}$ and Ca$^{2+}$, hypothetically leading to a decreased biofilm formation in HD catheters filled with TSC. Clinical studies showed that an interdialytic lock with TSC is more effective than interdialytic locking with heparin in reducing morbidity and mortality due to catheter-related infections [1,2,11]. Also, TSC as locking solution can prevent bleeding complications due to leakage of heparin from dialysis catheters [12,13].

Many dialysis centres in Europe have replaced heparin with TSC because of its benefits. So far, no clinically relevant side effects were reported during catheter locking with TSC. This is important, as concern has risen of using TSC for catheter locking after a report of a fatal cardiac arrest following the direct injection of 10 ml of a high concentration of TSC 46.7%. This incident led to the restriction of use of tricirosol in the USA [14]. In this particular case, however, a large amount of TSC was injected in a previously unstable patient with severe electrolyte disturbances. It is clear that the use of these solutions should be restricted to authorized and skilled health-care professionals.

Reduction of biofilm formation in HD catheters in vivo by using different interdialytic locking solutions has not been previously studied. We hypothesized that TSC catheter locking compared to heparin catheter locking may reduce the biofilm formation, explaining the effectiveness of TSC in reducing CRBSIs. Therefore, we compared the effect of TSC and heparin catheter locking solutions on biofilm formation and bacterial colonization in HD catheters in a randomized trial. We used standardized scanning electron microscopy techniques and performed standardized microbial cultures.

**Subjects and methods**

**Selection of patients**

The study was conducted from October 2007 until January 2008 in a dialysis unit of a teaching hospital in the Netherlands. On average, 100 HD patients are treated in our unit, 15% of the patients depend on a catheter for vascular access.

Patients were eligible for enrolment in the study when they were older than 18 years, were not admitted to the intensive care ward and experienced chronic or acute renal failure that required HD treatment by means of an HD catheter. Only patients with a newly inserted, well-positioned double-lumen precurved jugular HD catheter (15.5 FR Freeflow®, Medcomp, Medical Components Inc., Harleysville, PA, USA) that was expected to be needed for >2 months could be included.

Patients were excluded from the study if any of the following criteria were present: systemic or localized bacterial infection requiring systemic antibiotics and proven or suspected allergy to heparin or TSC. Written informed consent was obtained from all patients before enrolment.

**Study design**

Participants were randomly assigned to have their catheter locked with either TSC 30% (Dirinco BV, Rosmolen, The Netherlands) or unfractionated heparin 5000 U/ml (Leo Pharma, Breda, The Netherlands) for 1 month. After this month, all HD catheters were exchanged over a guidewire. Subsequently, the patient received the other locking solution, again for the duration of 1 month. Thereafter, the catheter was exchanged again.

After the catheter removal, the catheter was aseptically dissected into three segments: the subcutaneous segment from the insertion site (the hub), the middle segment and the distal part (the tip). Each segment was bisected into two longitudinal subsegments of exactly 10 mm. The entire internal surface of each half segment was examined by electron microscopy, and the other half was used for culturing.

All catheters were inserted by an experienced nephrologist under ultrasound guidance. The catheters were only used for HD treatment. After each HD treatment, the catheter lumen was flushed with 10 ml sodium chloride 0.9% and instilled with the prescribed solution. The volume used for each lumen was exactly equivalent to the volume noted on the catheter. Catheter care protocols were according to national guidelines for HD treatment. These guidelines included catheter exit site-dressing changes after each session and catheter manipulations only to be performed by trained dialysis staff wearing masks and sterile gloves. Use of dry gauze dressings and mupirocin 2% ointment at the catheter exit side was performed routinely.

**Quantitative examination by electron microscopy**

The hub, the middle segment and the tip were placed in a fixative solution consisting of 5% glutaraldehyde in cacodylatebuffer (0.1 M, pH 7.0) with 0.15% ruthenium red immediately after removal from the patient. The sections of the catheter were then ‘metallized’ with osmium tetroxide and thiocarbohydrazide. This was followed by dehydration in ethanol before critical point drying (Polaron SEM sputter coater, 1.4 kV, 18 mA, 20 s) and examining with a scanning electron microscope (SEM) (JEOL-JSM-6301F scanning microscope, voltage 3 kV).
For the quantitative calculations of biofilm distribution, specimens were examined by an SEM operator who scanned the entire inner surfaces of each section of the catheter at a magnification of ×180 to establish the proportion of each surface covered by biofilm. The average distribution of biofilm on the surfaces was quantitated as percentage of surface area covered by biofilm layer and was calculated by scanning the biomaterial surface with Scion Image Analysis (Beta 4.0.3, Scion Corporation, Maryland, USA).

Surfaces were subsequently examined at higher magnifications (×10 000) at regular intervals to ascertain if the amorphous biofilm layer actually contained visible bacteria: photographs were made of the bacterial cells and other features of the biofilm surfaces and compared with the aspect of an unused catheter. The electron microscopy operator was blinded regarding assignment to the patient and locking solution [15,16].

### Quantitative catheter cultures

Catheter cultures were performed in accordance with standardized culture techniques according to guidelines of the Centre of Disease Control. The catheter subsegments were rolled over a blood agar plate, a chocolate agar plate and a Schaedler agar plate and subsequently put in a brain heart infusion broth. All microorganisms were identified according to standard laboratory techniques in accordance with the Centre of Disease Control. Local catheter infection was defined as isolation of ≥15 cfu by the roll plate technique from the catheter segments [17].

### Statistical analyses

Statistical analysis was performed with SPSS software 13.1 (SPSS inc., Chigago, IL, USA). Calculation of the required sample size was based on the assumption that TSC would reduce the biofilm formation with 75%. With a two-sided test, an α level of 0.01 and a power of 95%, the analysis required six catheters per group. Continuous variables with normal distribution, such as the extent of biofilm on the surfaces of catheters, were compared by the Student's t-test. 

\[ P < 0.05 \text{ was considered to be significant.} \]

The results obtained from the catheter cultures were compared by the χ²-test. Differences were considered statistically significant for \[ P < 0.05. \]

### Results

A total of six HD patients were randomized, and 11 catheters were studied. One patient did not complete the second period because an arteriovenous fistula was ready for use. Thirty-two subsegments were retrieved from the catheters. One subsegment was damaged during the fixation procedure and therefore excluded in the final analysis. Patient characteristics are shown in Table 1. No serious adverse events or infectious periods were observed.

---

**Table 1.** Baseline characteristics of the 11 haemodialysis patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TSC started group (n = 6)</th>
<th>Heparin started group (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (range)</td>
<td>64.7 (33–81)</td>
<td>61.4 (33–75)</td>
</tr>
<tr>
<td>Male gender</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Time on dialysis (months)</td>
<td>2.5 (0.0–11.0)</td>
<td>3.8 (0.0–12.0)</td>
</tr>
<tr>
<td>Cause of ESRD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Renovascular</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 2.** Distribution of biofilm was quantitated as percentage of total surface area covered by biofilm layer measured by Scion Image analysis

<table>
<thead>
<tr>
<th>Mean distribution of biofilm</th>
<th>TSC subsegments (n = 17)</th>
<th>Heparin subsegments (n = 15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subsegment</td>
<td>Hub 17%</td>
<td>87%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mid 7%</td>
<td>45%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tip 23%</td>
<td>57%</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>16%</td>
<td>63%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean distribution of biofilm.

For the quantitative calculations of biofilm distribution, specimens were examined by an SEM operator who scanned the entire inner surfaces of each section of the catheter at a magnification of ×180 to establish the proportion of each surface covered by biofilm. The average distribution of biofilm on the surfaces was quantitated as percentage of surface area covered by biofilm layer and was calculated by scanning the biomaterial surface with Scion Image Analysis (Beta 4.0.3, Scion Corporation, Maryland, USA).

Surfaces were subsequently examined at higher magnifications (×10 000) at regular intervals to ascertain if the amorphous biofilm layer actually contained visible bacteria: photographs were made of the bacterial cells and other features of the biofilm surfaces and compared with the aspect of an unused catheter. The electron microscopy operator was blinded regarding assignment to the patient and locking solution [15,16].

### Quantitative catheter cultures

Catheter cultures were performed in accordance with standardized culture techniques according to guidelines of the Centre of Disease Control. The catheter subsegments were rolled over a blood agar plate, a chocolate agar plate and a Schaedler agar plate and subsequently put in a brain heart infusion broth. All microorganisms were identified according to standard laboratory techniques in accordance with the Centre of Disease Control. Local catheter infection was defined as isolation of ≥15 cfu by the roll plate technique from the catheter segments [17].

### Statistical analyses

Statistical analysis was performed with SPSS software 13.1 (SPSS inc., Chigago, IL, USA). Calculation of the required sample size was based on the assumption that TSC would reduce the biofilm formation with 75%. With a two-sided test, an α level of 0.01 and a power of 95%, the analysis required six catheters per group. Continuous variables with normal distribution, such as the extent of biofilm on the surfaces of catheters, were compared by the Student's t-test.

\[ P < 0.05 \text{ was considered to be significant.} \]

The results obtained from the catheter cultures were compared by the χ²-test. Differences were considered statistically significant for \[ P < 0.05. \]
The average distribution of biofilm on the surfaces of the two groups is shown in Table 2. Ultrastructural colonization and biofilm formation were present in all catheters. Microbial organisms consisting mostly of coccal forms buried in a biofilm layer could be visualized in some degree on the surface of almost all HD catheters examined. Typical examples are shown in Figures 1 and 2.

In HD catheters locked with TSC, an average coverage by biofilm of 16% (±SD 6.8) was found. Catheters locked with heparin resulted in a significant larger amount of biofilm coverage (63%; ±SD 22.9; \( P < 0.001 \)). No significant differences were found between colonization in the hub, mid and tip subsegments (Table 2).

Of the total of 32 subsegments, 11 (34%) demonstrated positive cultures. Of the catheters who were randomized to heparin locking, eight of 15 subsegments showed positive cultures (53%) compared to three of 17 subsegments of catheters who were assigned to TSC (18%; \( P < 0.05 \)) (Table 3). Staphylococcus epidermidis was the organism most frequently cultured (45%).

**Discussion**

This is the first randomized controlled trial to demonstrate in vivo (with SEM) that the internal surface of catheters locked with TSC had significantly less microbial biofilm per surface area than catheters locked with heparin. In HD catheters filled with TSC, an average coverage by biofilm of 16% was found versus a 63% in catheters locked with heparin. Furthermore, less positive cultures were retained from segments from catheters that had TSC as locking solution.

We could demonstrate that the choice of catheter lock solution is likely to have a significant effect on the ability of bacteria to adhere to the surface of central venous HD catheters. Only a few comparative studies have been performed studying the influence of TSC and heparin on catheter-related biofilm formation [8,10,18,19]. Also, the superior antimicrobial properties of a new catheter lock solution containing citrate 7% in combination with methylene blue and parabens (C/MB/P) over heparin were recently shown in an in vitro study of Steczko et al. Solutions containing C/MB/P resulted in a reduction in biofilm formation and killing bacteria embedded in an existing biofilm [20].

However, very limited in vivo data on the antimicrobial properties of TSC as HD catheter locking solution are presently available.

Considering possible mechanisms, it is likely that the most important effect of TSC is chelation of the divalent cations Ca\(^{2+}\) and Mg\(^{2+}\). From dentistry research, it is known that a Ca\(^{2+}\)- and Mg\(^{2+}\)-chelating agent like TSC inhibits the growth and coaggregation of microorganisms and may prevent the formation of a biofilm that consists of microbes in a firm glycocalyx [10]. A reduction of the incidence of catheter-related bacteraemia by the intraluminal route could be the result. This hypothesis was tested in some in vitro models with catheter segments but the constructions with catheters or fragments trying to imitate the clinical situation are artificial [21,22].

Colonization of the catheter itself is not sufficient to lead to a clinical important infection. Although in all catheters some degree of biofilm formation was demonstrated, only 11 of 32 catheter subsegments showed positive cultures, and no patient developed a CRBSI. These findings are consistent with other studies with electron microscopy of medical devices such as cardiac pacemakers and urinary devices [16].

| Table 3. Relationship between biofilm coverage and culturing results |
|-----------------------------|-----------------------------|
| TSC  | Heparin |
| n    | Biofilm coverage | n    | Biofilm coverage |
| Positive culture | 3 | 12% | 8 | 68% |
| Negative culture | 14 | 17% | 7 | 57% |

There was no significant difference in distribution of biofilm between catheter subsegments of catheters associated with positive cultures versus culture-negative subsegments (Table 3).
Reduction of biofilm formation with trisodium citrate

As host factors like humoral and cellular response and leukocyte function are also an important defense mechanism against CRBSIs; larger in vivo studies are needed to correlate biofilm formation to CRBSIs. However, as clinical studies have already shown clinical superiority of TSC over heparin, it is not very likely these studies will be conducted in the future.

Subject of discussion remains the optimal concentration of citrate in catheter lock solutions. It is known that the efficacy of citrate is dose-dependent with the best results for high concentrations. The last several years, many dialysis departments have experienced the benefits of TSC 30% without reports of major complications. Catheter locking solutions containing citrate in combination with other agents are promising. Although, large clinical studies should be performed to determine the effects of these solutions in vivo.

Interestingly, there was no significant difference between biofilm surface area coverage in the hub, mid and tip sub-segments. Apparently, biofilm formation is a rapid process, as within a period of 1 month, all HD catheters showed some coverage by a microbial layer in any segment. This is in agreement with the results in experimental studies. Cooper et al. has demonstrated that bacteria inoculated at the exit side of a subcutaneous plastic catheter migrate rapidly and can be detected 4 cm from the entry side within 1 hour [23]. Although the median catheter use of 30 days in our study is relatively short, luminal colonization is noted to become predominant within 30 days of catheter placement [16]. However, this study does not provide data about the influence of time on progression of catheter luminal coverage with biofilm. Additional studies should explore the effect of locking solutions on the biofilm formation in HD catheters with longer periods of catheter use to estimate the time as factor of influence.

There are some limitations to our study. We only used a small number of patients. Nevertheless, the statistical power was considered sufficient, and the differences in both groups were evident. Furthermore, the major goal of study could be accomplished.

Using SEM for studying biofilms can be difficult. Calculating the percentage of the surface area covered by biofilm can be hampered by the varying nature of the biofilm. We used the presence of bacteria at higher magnifications as the main criterion to term the glycocalyx a biofilm. Furthermore, we used a standardized SEM technique, and the investigators were blinded to the locking solution assignment. Therefore, it is likely that our study results are valid.

In conclusion, our study demonstrates an in vivo reduction of intraluminal catheter biofilm formation and bacterial colonization with the use of TSC 30% for HD catheter locking. By this mechanism, prevention of catheter-related infections by TSC can be largely explained.

Conflict of interest statement. None declared.

References


Received for publication: 20.06.09; Accepted in revised form: 6.11.09