Is biofilm a cause of silent chronic inflammation in haemodialysis patients? A fascinating working hypothesis

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Introduction

Knowledge of biofilm formation and its biological role in chronic subclinical inflammation has largely evolved over the past years [1–5]. The development of a biofilm is a very effective way for bacteria to survive facing hostile conditions and to resist biocides and
antimicrobial substances. The initial event in biofilm formation is the adhesion of microbes to surfaces. The surface properties of medical devices are usually modified by a conditioning film of organic material. The effect of single blood proteins or of whole blood itself depends on bacterial strains. Fibrinogen and fibronectin both enhance *Staphylococcus aureus* binding and inhibit *Staphylococcus epidermidis* or Gram-negative bacteria adherence, while whole blood promotes *Pseudomonas aeruginosa* biofilm formation [6].

Biofilm is not a static simple matrix made of homogeneous slime embedding bacteria. This sessile multicellular community is a dynamic complex system made of exopolysaccharide matrix embedding living microorganisms with a phenotype modified from planktonic (free-floating) bacteria. Biofilm is a living material that evolves according to local microenvironmental conditions (hydrodynamic and biochemical conditions, thickness, shear stress and possibly others). Biofilm has a spatial heterogeneity (channels, towers) that seems to be linked to the type of bacteria and may differ in relation to oxygen limitation, pH, nutrient access and growth rates.

However, despite these important acquisitions, the role of biofilm in haemodialysis patients is undefined. At present, we are unable to define the extent and the incidence of biofilm formation in the haemodialysis population. No method exists to detect biofilm in vivo and to ascertain its eradication. In principle, bacteria are often shown to form biofilm using ‘extreme’ and unrealistic experimental conditions. Detection of biofilm is usually performed by surface ultrastructural analysis alone or in combination with methods to stain the bacterial nuclear DNA using membrane-permeable or impermeable fluorochromes (to detect dead or live bacteria, respectively). In this editorial, we will describe several potential sites of bacterial contamination and possibly of biofilm formation in haemodialysis patients, such as the vascular access (arterio-venous fistula and graft or venous catheters) and the hydraulic circuit of the water treatment and of haemodialysis monitors.

**Vascular access in haemodialysis patient**

Vascular access offers an excellent way for bacteria to invade the bloodstream of haemodialysis patients. Vascular access (arteriovenous or venovenous) represents a door open to the internal milieu three times a week. Arteriovenous vascular accesses (native arteriovenous fistula and synthetic graft fistula) are usually located superficially under the skin, close to an area where bacteria proliferate quite easily. Needle insertion breaks the skin barrier and facilitates the penetration of topical bacteria into the bloodstream. Bacteria may circulate either as a planktonic form to be finally cleared by the reticuloendothelial system (particularly in the lungs and spleen) or they may adhere to the vascular endothelium or to a foreign surface (e.g. synthetic prosthetic graft or catheter polymer). At this stage, immobilized bacteria modify their phenotype and start producing slime matrix embedding living bacteria [7]. In principle, this stage represents the first step of biofilm formation. Native arteriovenous fistulae are obviously less exposed to biofilm formation than synthetic materials [8]. In most cases, the penetration of bacteria into the bloodstream will induce an acute febrile event. An intact vascular endothelium possesses efficient host defence mechanisms capable of preventing adhesion and of neutralizing adherent bacteria. Stagnant zones are often associated with an altered endothelium and partial thrombosis, such as in the case of a ‘false aneurysm’. Here, bacteria may adhere to the endothelium, possibly forming biofilm. Thus, an inflammatory ‘false aneurysm’ on an arteriovenous fistula should always be removed under antibiotic therapy. On the other hand, synthetic graft material (e.g. PTFE) or central venous catheters made of synthetic polymer (polyurethane or silicone rubber) are clearly more exposed to bacteria [9]. Synthetic materials have none of the anti-adhesive or anti-bacterial properties of the endothelium. The material surface usually consists of crevices and cracks that facilitate adhesion of bacteria. Moreover, synthetic materials promote the adsorption of circulating proteins (albumin and clotting proteins) on their surfaces, thus enhancing bacterial adhesion and proliferation. Flow stagnation in venous catheters, which occurs during the inter-dialytic period, may offer ideal conditions to immobilized bacteria for proliferation and biofilm formation.

As we stated above, there is no method to detect the presence of biofilm. Even if biofilm could be detected subsequently by surface morphological analysis, biofilm in the vascular access may have remained clinically asymptomatic. Occasionally, it may be suspected by the sudden onset of a febrile reaction occurring during a haemodialysis session in the absence of overt signs of infection. This type of reaction illustrates the fact that bacteria may be released from the biofilm, possibly in the presence of facilitating factors such as a high blood shear stress leading to *de novo* bacteremia. Biofilm is the source of chronic, subclinical inflammation due to the repeated stimulation of monocytes/macrophages. The release of pro-inflammatory cytokines may not only provide a strong stimulus for a sustained acute phase response, increased plasma levels of C-reactive protein and erythropoetin resistance, but may also favour activation of the coagulation pathways leading to vascular access thrombosis [10,11].

Accordingly, it is of paramount importance to prevent the penetration of bacteria into the bloodstream at the time of connection (or disconnection) of the patient to the haemodialysis monitor. This risk underlines the critical role of nursing care and clinical practice in managing the vascular access and in preventing bacterial contamination [12,13]. PTFE graft vascular accesses are more exposed than native fistulae to bacterial contamination, the relative risk
being estimated at around three times higher [14]. Interestingly, such a risk persists in thrombosed PTFE prosthesis. It is therefore recommended to remove an unused graft to prevent chronic inflammation in haemodialysis patients [14]. Venous accesses (catheters and port devices) are more exposed to bacterial implantation. Biofilm formation is likely to occur in prolonged stagnation (e.g. during the inter-dialytic period). This risk is clearly illustrated by the fact that the incidence of symptomatic infections is seven times higher with catheters than with native arteriovenous fistulas [15]. Moreover, clots formed at the tip of the unused catheter during the inter-dialytic period provide a perfect nutrient medium for bacterial proliferation and possibly biofilm formation. Interestingly, it has been shown that the regular use of a catheter locking solution, with a dual action antithrombotic and antimicrobial venous port catheter device, prevents the formation of biofilm (W. Costerton, personal communication) and reduces the incidence of bacteraemia [16–18]. Dual action antithrombotic and antimicrobial locking solutions offer an attractive means to prevent catheter colonization and biofilm formation in permanent catheters providing the safety of the solution is ensured [19]. Catheter locking solutions deserve further control studies in permanent catheters before they can be proposed as a routine pre-emptive therapy for catheter-related morbidity [20].

**Haemodialysis monitors**

The highest risk of bacterial contamination and biofilm formation in haemodialysis monitors may derive from the water tubing connecting the reverse osmosis–water distribution loop with the individual haemodialysis monitors. In this critical part of the system, a biofilm may well form in the stagnant phases (e.g. during the night). These connecting lines need to be included in routine disinfection procedures and replaced on a regular basis. In order to reduce biofilm formation, disinfection of the entire water distribution system, including the connecting tubes and the dialysis monitor, is required on a regular basis. Regularly means at least weekly, better daily. Disinfection can be done using chemicals, ozone or heat [21,22]. Pyrogens released from biofilm include not only endotoxins but also bacterial DNA fragments which are small, trigger Toll-like receptors on monocytes and induce cytokine production leading to an inflammatory response [23]. Bacterial DNA fragments are not removed from contaminated dialysate by ultrafiltration. Therefore, installation of ultrafilters in the dialysate line does not replace the need for disinfection of the entire water distribution system.

Haemodialysis monitors represent the final link of the water system chain transforming tap water into dialysate. Haemodialysis monitors have additional reasons that expose them to bacterial proliferation and biofilm formation: the presence of basic and acid concentrate solutions and inlets, dialysate connectors and the drain itself.

Once contamination has occurred, biofilm formation depends on the physical properties (i.e. design, length and water flow velocity) and on the material surface properties (polymer and roughness) of the hydraulic circuit (Figure 1A and B). Disinfection is the only way to prevent bacterial proliferation. However, all disinfection protocols are validated in relation to microbial killing and not to biofilm prevention and eradication [22,24].

According to medical device regulations, it is the manufacturer’s responsibility to determine proper disinfection methods and protocols. In routine practice, non-compliant or inadequate operative protocols may lead to inappropriate disinfections. Re-contamination may occur during technical maintenance controls. Re-contamination may also derive from a persisting bacterial focus, depending on the mode of disinfection, technical design of the machine, duration of haemodialysis and flora in the dialysate [25,26]. A stable microbial contamination causes biofilm, and biofilm itself decreases disinfection treatment effectiveness on both bacteria and endotoxins [5,21,23,26–28].

A high level of decontamination for haemodialysis machines is recommended nowadays when ultrapure dialysate is required not only for on-line treatment but also for standard dialysis. Ultrapure dialysate with a final ‘on-line’ dialysate ultrafiltration represents a quality assurance method and enables high standard levels of purity [25].

Quality controls and surveillance programmes are of paramount importance to ensure production and delivery of an ultrapure dialysate. Disinfection procedures may be validated from results obtained through a regular monitoring of incoming water and dialysate. The most recent guidelines stress all these aspects and introduce the concept of quality assurance in dialysis to ensure patient safety [26].

**Conclusions**

Despite the large degree of uncertainty as to the incidence of biofilm formation in haemodialysis, several sites are definitively exposed to bacterial contamination and proliferation. If biofilm is formed, it would be very resistant to conventional disinfection methods. At present, all efforts should be made to prevent bacterial contamination and colonization. New methods are expected and required to help in detecting biofilm formation in the vascular access and dialysate tubings in order to prevent and to treat inflammatory complications in haemodialysis patients.

Future studies hopefully should define whether biofilm formation may be a cause of chronic inflammation—often a ‘silent’ (subclinical) process. This is a new challenge to establish haemodialysis
adequacy. It will introduce a new dimension in the treatment, i.e. dialysis quality adequacy.

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References


Fig. 1. (A) Bacteria adhering onto the surface of a haemodialysis tubing and starting the formation of a biofilm. (B) Bacteria and biofilm trapped in a crystal network made of calcium and magnesium carbonate salt in a dialysis tubing.
Are PD patients with or without residual renal function qualitatively different—or are they simply at different stages of the continuum of progressive uraemia?

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The value of residual renal function to patients treated with peritoneal dialysis (PD) is beyond dispute. CANUSA was the first study to demonstrate this, initially expressing the benefit in terms of small solute clearance in combination with peritoneal clearances, although on re-analysis this benefit could be reduced to the simplest of all measures—residual urine volume [1,2]. Nevertheless, CANUSA is often misrepresented. It is the preservation of renal function, not its initial