Designing a filter to prevent infections with spore-forming bacteria in Heroin-injecting drug users

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Background

In heroin users, there have been a number of outbreaks caused by spore-forming bacteria, causing infections such as anthrax or botulism (Fig 1). In 2000, there were 60 confirmed cases of *Clostridium novyi* infection amongst IDUs in Scotland, with an 87% fatality rate [1]. In 2009/10, there was a notable outbreak with 119 cases of injectional anthrax, and 19 deaths were reported in the UK and Germany. These outbreaks are, most likely, caused by injecting heroin contaminated with bacterial spores.

Fig 1. Severe illnesses resulting from the injecting heroin contaminated with spore-forming bacteria of the *Clostridium* and *Bacillus* species.

Aims

Our aim was to develop a filter that: (a) efficiently removes both particulates and bacterial spores; (b) has a low hold-up volume (the dead volume of the filter); (c) fast filtration time; and (d) also lead to minimal losses of the active ingredients in heroin.

Methods

- ***Heroin sample preparation***

0.1 g of brown heroin was suspended in 0.7mL distilled water with 50 mg of citric acid. Then, using a Bunsen burner, the solution was heated until it just started to boil and became clear. Volume was adjusted to 0.8 mL.

- ***Bioburden testing***

Seven heroin samples were tested for their bioburden using a viable counting method. Amplification and sequencing of the 16rRNA gene from ten randomly chosen colonies was used to identify the bacterial species.

- ***Filtration using existing devices and membranes***

Filtration of samples was performed with the following filter devices: 25mm Minisart RC25 syringe filters (0.2µm pore size, Sartorius), 15mm Minisart RC15 (0.45µm pore size, Sartorius), 13 mm Millex-GV (0.22µm pore size, Millipore), and 4 mm Millex-GV (0.22 µm pore size, Millipore), and a Swinnex filter holder (fitted with a 13 mm membrane, Millipore).

- ***Design of a novel filter***

This device was made from polycarbonate , and made by machine tooling in the local workshop in the University of Bath.

- ***Filtration time of the prototype filtration device***

We assembled inside the prototype filter the glass microfiber prefilter with different 0.2µm membrane filters. The filters tested were polyvinylidene fluoride (PVDF), polytetrafluorethylene (PTFE), mixed cellulose esters (MCE), polyethersulfone (PES) and Nylon.

- ***Binding of active ingredients in heroin to membranes***

The samples were analysed before and after filtration using an UHPLC-MS/MS system.

- ***Removal of bacterial spores using the prototype filtration device***

A 0.8mL heroin sample was spiked with *Bacillus subtilis* spores (~10^8 spores), followed by filtration. Samples were then plates on agar plates, and the number of colonies counted after overnight incubation at 37°C.

Results

- ***Bioburden testing***

Aerobic endospore-forming bacteria (*Bacillus* spp) were the predominant microflora isolated. The largest number of colonies were obtained on brain heart infusion agar plates, with a bioburden of 580 CFU/g. Three colonies were identified as *B. licheniformis*, two as *B. pumilus*, two as *B. subtilis*, one as *B. thermolactis*, one as *B. massiliosenegalensis* and one colony was identified as *Staphylococcus hominis*,

- ***Why are existing bacterial filters are not suitable?***

Losses ranged from 7.5% (13 mm Millex GV filter) to 44% (Minisart RC25 (25 mm) filter (Fig 2). These were due to some of the heroin being left in the filter housing, thus representing the hold-up (or dead) volume. In case of the 4 mm Millex GV filter, it was not possible to filtrate the sample as the filter immediately blocked up, due to the presence of particulate matter in the heroin.

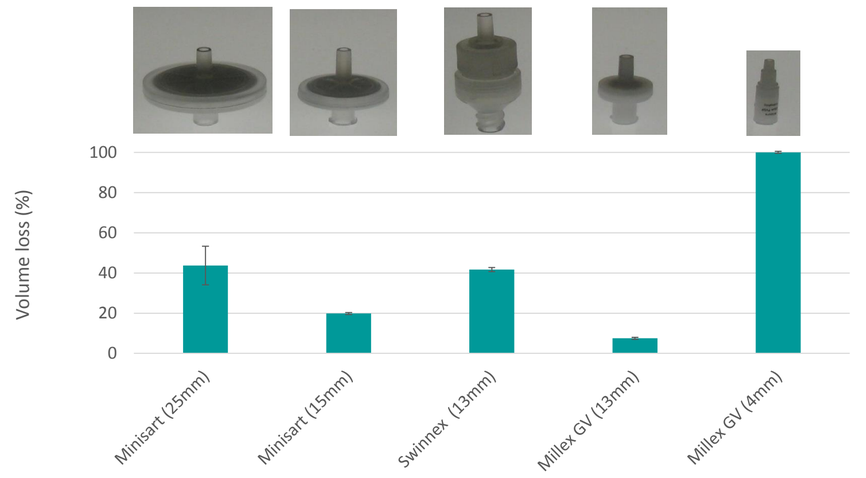


Fig 2. Evaluating the loss of volume in a 0.8 mL heroin sample by filtration using commercially available syringe filters. The Swinnex filter holder was fitted with a 13mm PVDF membrane.

- ***Design of a novel filter***

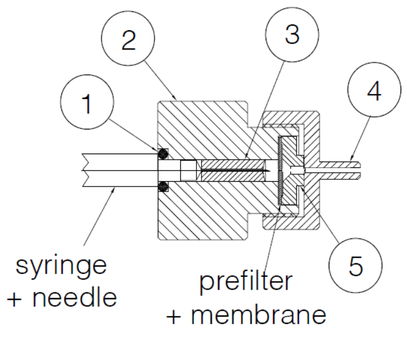
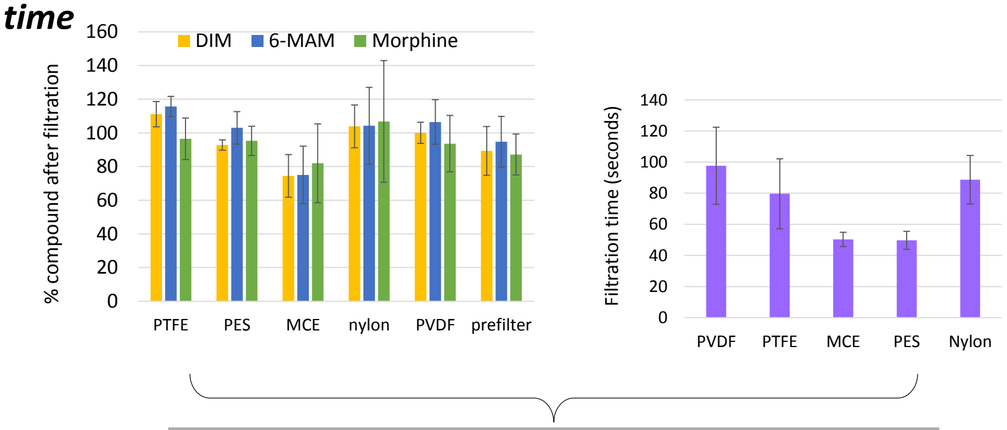


Fig 3. A sketch showing the design of a prototype filter which allow for upward filtration, filter particulate and spores in one step. Figure 3. Filtration device. The device is assembled from 5 parts: (1) butyl rubber O ring; (2) syringe holder; (3) needle collar; (4) filter inlet; and (5) membrane plate.

As shown above (Fig 3), the filtration device was designed so that it could accommodate insulin-type injection syringes with a fixed needle and do the filtration in one easy step while drawing the heroin solution upward. A 2 µm glass fibre prefilter can prevent blocking of a 0.2µm filter by particulate matter. Due to limitation of the tooling equipment the device is rather bulky, but we envisage that the filtration device will be significantly smaller in the final design.

- ***Effect of membrane filters on active ingredients in heroin – Filtration time***



In Fig 4, PES does not bind active ingredients (left graph) and shows shorter filtration time (right graph)

Fig 4. (Left) The percentage of active ingredients after filtration using different membrane filters. (n = 3). For all ingredients, the amount before filtration was normalised to 100%. (Right) Filtration time using different membranes filter.

- ***Removal of bacterial spores using the prototype filtration device***

We tested the capability of removing bacterial spores from heroin. This was successful, as no growth was observed after filtration. Thus, even though the bacterial load added was high, no spores passed through the filter showing efficient removal of the spores.

Conclusions

Infections in IDUs are common, with one potential source of bacterial pathogens being the heroin itself. In this paper, a filtration device is developed that removes bacteria and particulates. Such a device could be provided to attract IDUs to existing needle and syringe exchange programmes across Europe and beyond. Their overall aim is harm reduction.

Conflict of Interest

The authors declare that they have no competing interests

Acknowledgments

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References

[1] McGuigan CC *et al* (2002) *J Med Microbiol* 51 971-977.