

Review of: "Sonochemical coating of Prussian Blue for the production of smart bacterial-sensing hospital textiles"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

This study investigates the general applicability of a Ferric Hexacyanoferrate Nanoparticles (Prussian Blue) textile coating to detect metabolic-active bacteria. The sensing capacity of the coating bases on a redox reaction: The textile coating turns from blue to white mediated by the metabolic activity of bacteria. The reaction is reversible, the textile can be recovered to blue again by oxidation either by oxygen (air, solution) or by an oxidizing agent such as H_2O_2 . As a future field of application, hospital textiles are mentioned, and the prevention of hospital-acquired infections (HAI) is pointed out as a potential benefit. In a first part of the study the preparation and optimization of the Prussian Blue coating by a sonochemical process was described and the redox capacity was proven. Second, the colour loss in different media and buffer solutions without test microorganisms was investigated to choose a suitable buffer solution with a low decolorization potential for the follow-up experiments. Third, coated textiles were incubated in a bacterial solution with either *Escherichia coli* or *Staphylococcus aureus* for up to 120 hours to assess the bacterial sensing potential of the coated textiles. Fourth, cytotoxicity of different Prussian blue concentrations and the antimicrobial action of coated textiles were investigated.

The study shows that Prussian blue coated textiles can detect metabolic-active bacteria by a visible colour change from blue to white. However, the reaction of the coated textile has so far only been investigated on textiles immersed in a buffer-solution with *E. coli* or *Staphylococcus aureus*. A colour-change visible by the naked eye, was detectable after ca. 6 to ca. 15 hours. The colour loss was highly dependent on 1) the initial microbial counts which needs to be very high for a visible reaction (around $1E+09$ cfu/ml); 2) the used medium with the presence/absence of specific ions or a certain pH; 3) the incubation time with the reduction reaction by the metabolic activity of the test microorganisms on one hand and the oxidation by oxygen in the medium on the other hand, 4) the type of bacteria, with a higher and faster colour loss for gram-negative *E. coli*.

Textiles can play an important role in infection transmission in the hospital field. A simple, equipment-free method to detect a critical microbial contamination of textiles is so far not available and the present approach is therefore of great interest. However, this study investigates the applicability of Prussian Blue coating on a basic level. The transferability of the findings to the field of application is only partly

addressed and discussed.

There are several concerns and open questions with this study which include the following:

1. The title indicates that a bacterial-sensing textile coating is applicable for hospital textiles, whereas the study provides only a general proof of concept of the textile coating without transferring the findings to the described field of application.
2. It is not clear if negative controls (colour loss in buffer solution without test bacteria) were included when the bacterial sensing activity of coated textiles was assessed. A potential impact on the colour change of the coated textiles due to the buffer solution itself, or the oxygen diffusion into the solution after a longer incubation time should be quantified and subtracted from the test results.
3. The response time and sensitivity of the coating was only investigated with textiles immersed in bacterial solutions. Can a colour change also take place on dry textiles? As the colour-change is mediated by some proteins and other mediators of the bacterial electron transport chain, it can be argued, that under dry conditions, the reduction reaction might be decreased due to the lacking contact of the coating with the microorganisms, respectively the absence of a solvent (water). Furthermore, oxygen in the ambient air might react with the coating and biases the bacterial-sensing reaction. The impact of ambient conditions such as temperature and relative humidity might play a further role on the sensing capacity of the coating.
4. The sensitivity of the coating is with 10^9 cfu/ml bacteria for a visible reaction low. It can be questioned, if such a low sensitivity really helps to prevent HAIs?
5. The response time with several hours to days is long and it can be questioned if this really helps to prevent infection transmission.
6. Due the limitation to metabolic active bacteria, bacterial spores can't be detected with the Prussian Blue textile coating. The spore forming *Clostridium difficile* causes the most prevalent HAI (Lessa, Mu, et al., 2015; He & Miyajima, 2012) and beside other vectors, textiles are suspected to play an important role in its transmission (Lessa & Mu, 2015).
7. Is the coating washable? Can the chemistry in the washing process help to recover the colour?

Literature

He, M., Miyajima, F., et al., *Nature Genetics*, **45(1)**, 109-113 (2012).

Kumar, N., Miyajima, F., et al., *Clinical Infection Diseases*, **62(6)**, 746-752 (2015).

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