How a new antimicrobial bacteriological barrier multiplies BSC safety levels

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Introduction and objectives

The laminar airflow cabinets are key equipment in research centres, universities, or healthcare facilities. Their function, depending on the model and the application can either be protection of the operator while handling potent compounds or, in other cases, protect the product itself.

In the field of laminar air flow cabinets, and in the pursue of increasing the operation safety from both perspectives, a coating based in nanotechnology and Titanium dioxide has been studied. Titanium dioxide as a photocatalytic material has been extensively studied and its properties as a biocide are reported by several studies, such as the one published by Abreu et al., (2013) [1].

The presented investigation is based on the ZeroCoat® patented technology, that intends to innovate throughout a new deposition process, where any metal peroxide can be adhered to a surface, leaving an invisible and colourless trace. The nanoparticles (<10 nm) created during these processes are highly effective and require less activation than other photocatalytic materials.

In order to understand the coating biocide capabilities, Telstar together with the Center for Innovation, Research and Transfer in Food Technology (CIRTTA) and the Universitat Autonoma de Barcelona, decided to challenge the nanotechnology proposed, ZeroCoat®, and compare it with the current biocide technology included in the Biosafety cabinets commercialised by the company, UVc light.

2. Material and method

A natural bioburden is usually composed by different species such as Staphylococcus, Pseudomonas, Klebsiella, Enterobacter or Bacillus (Larson et al., 2003). In order to get such a natural contamination a swabbing with Trypticase Soy Broth (TSB) in hands was done. This broth was then incubated during 24h at 30ºC.

After 24h, a second cultivation was done in TSB to get a population of at least 109 CFU/mL and ensure as well, that the microorganisms are in their growth phase, where the resistance is at its higher value. This media was eventually diluted to 1:100.

Ten stainless steel plates were prepared. They were divided in three equal parts, to ensure that each of them, would represent a different time of analysis. The measurements were done at 0, 2 and 12 days from the start of the treatment. Every plate was contaminated with 3 mL, homogeneously distributed by vertical, horizontal, and diagonal movements.

The ten plates were exposed to three different conditions:

- ZeroCoat plus UVc light (254 nm wavelength)
- UVc light, 254 nm wavelength (No coating)
- ZeroCoat plus LED light

Table 1 and Figure 2 show the distribution per condition and the real conditions of the plates.

Table 1: Stainless steel exposure conditions.

Plate	Treatment	Light conditions
1	ZeroCoat	UVc
2	ZeroCoat	UVc
3	ZeroCoat	UVc
4	No Coating	UVc
5	No Coating	UVc
6	No Coating	UVc
7	ZeroCoat	LED
8	ZeroCoat	LED
9	ZeroCoat	LED
10	No coating (Control)	No light

Figure 2: Telstar cabinet and plates distribution.

1. Microbiological analysis

The different portions of the plate were swabbed with peptone water and remained refrigerated until its analysis in the laboratory. Every tube was mixed for 30 seconds and then incubated with TSA, at 30ºC during 48h. To determine if the lethality was total, even the tubes with peptone water were incubated.

The results were shown in log (CFU/100 cm^2) and the effectiveness was expressed as log (N0) - log (Nt), were N0 is the initial count of microorganism, and Nt, the counting at 2 and 12 days.

2. Statistical analysis

The statistical analysis was done with the t-student method and applying an Analysis of Variance (ANOVA) to analyse the significant differences in between samples. The statistical package IBM-SPSS 22.0 was used.

3. Results and discussion

Table 2 and *Figure 3* show the results of the microbiological counting and the obtained lethality. Even if the initial counting was expected to be higher than 105 CFU/cm^2, the effect of the dilution together with the stress produced by the drying process, ended up in a count close to 105, but in all cases below this level. However, this contamination is still extremely high compared to real conditions and are considered, a worst-case scenario.

Table 2: Results of coating effect over the bioburden (Total mesophile aerobic microorganisms per cm^2).

Plate	Treatment	Light conditions	Days 0	Days 2	Days 12
1	ZeroCoat	UVc	4,06	0,00	0,00
2	ZeroCoat	UVc	3,68	0,00	0,00
3	ZeroCoat	UVc	4,44	0,00	0,00
4	No Coating	UVc	4,05	1,84	1,28
5	No Coating	UVc	3,78	2,16	0,51
6	No Coating	UVc	4,19	1,57	1,52
7	ZeroCoat	LED	4,92	0,00	0,00
8	ZeroCoat	LED	3,92	0,00	0,00
9	ZeroCoat	LED	4,27	1,73	0,00*
10	No coating (Control)	No light	3,58	2,35	2,33

The effect of ZeroCoat with UVc light was the most effective, obtaining a complete deactivation after 2 days of exposition. In the case of ZeroCoat with LED light, the deactivation was not total in one of the samples. It showed growth even after 12 days, as the counting showed was below the detection limit. After incubation during 48h, growth was encountered.

The plates not treated with coating, and only exposed to UVc light, only 2 logarithmic reductions were obtained, and no total reduction was achieved even after 12 days.

The control plate only showed a reduction of 1,2 logarithms due to the drying process that occurred during that time.

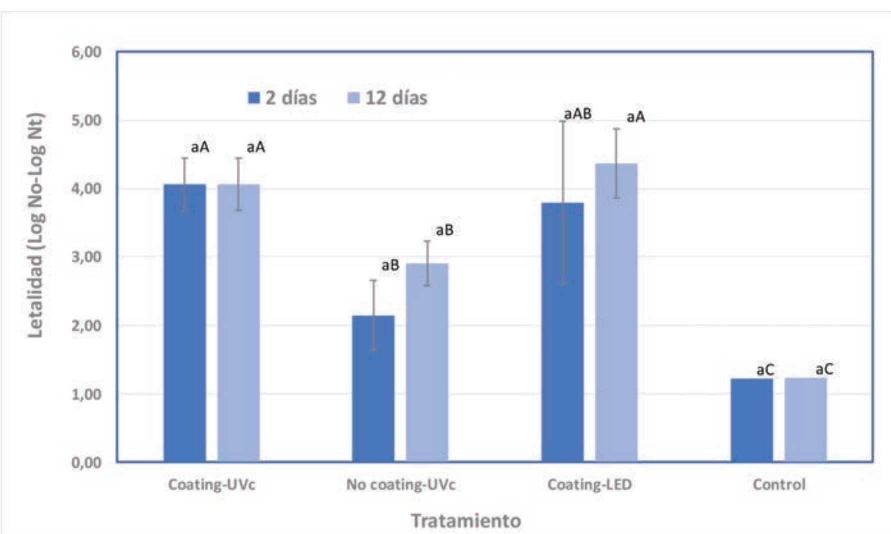

Figure 3: Lethality per treatment.

- Different capital letters show significative differences between the different treatments (p < 0,05)

- Different lower case show significative differences within the same treatment (p < 0,05)

The higher lethality in surfaces treated with ZeroCoat clearly shows that the photocatalysis has taken place even in conditions where the wavelength is not in the biocidal range. When the titanium dioxide is radiated with light, a photo-generation of holes in the surface is created.

These holes are highly oxidising and when water meets the surface, powerful ions are created. Hydroxyl or superoxide ions are then the main active agents attacking the organic matter surrounding the surface (Fujishima and Zhang, 2006) [2].

The effectiveness of UVc technology, even if the biocidal properties are broadly studied (based in the damage of the DNA of the microorganism), is very dependent not only in the distance from the source but also from the obstacles that may reduce the impact of the light (Otter et al., 2013) [3].

4. Conclusions

It is proved that the addition of a photocatalysis coating, such as the one offered by the ZeroCoat technology, decreases the overall bioburden of the equipment.

The surfaces treated with ZeroCoat and exposed to UVc are the ones with the best deactivation capabilities. In addition, the results obtained from the ZeroCoat treated surfaces, together with LED light are worthy to be considered as an alternative due to the obvious simpler and safer properties of the LED versus UVc light.

Finally, it is important to consider that the results of the UVc itself are very dependent on the status of the light itself as well as the potential contamination, obstacles or particles that interfere in between the source and the surface to be treated.

5. References

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Mireia Capella holds a Bachelor's Degree in Chemical Engineering from the Universitat Politecnica de Catalunya as well as a Master of Engineering in Chemical Processes from the Institut Quimic Sarria. After more than 3 years working as a R&D Project Engineer in Telstar, Mireia has actively contributed to process related projects, being the hydrogen peroxide technology development and validation her main domain of expertise. Due to her academic background and her continuous involvement she has been able to lead the launching of the ionHPlus technology at Telstar.

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