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To: Advanced Materials – JTJ, Inc.
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Results of testing the efficiency of paint containing TiO_2 using a microbiological method

According to the order of company Advanced Materials – JTJ, Inc., 273 01 Kamenné Žehrovice 23, Company ID no. 26763842, VAT Company ID no. CZ26763842 dated on April 24, 2008, represented by Jan Procházka,, antibacterial tests of photocatalytic paints were performed using materials, which were handed over during the meeting on April 22, 2008.

Methodology

1. Two kinds of bacteria, which survive on surfaces in medical facilities, were used:
   Staphylococcus aureus, tribe CCM 3953
   Enterococcus faecalis, tribe CCM 4224

   We use both tribes to test the efficiency of germicides with the suspension micro method. They are sufficiently resistant for testing antimicrobial substances.

2. The density of bacteria in a suspension was measured with a densitometer and it exceeded $1 . 10^{8}$ CFU per 1 ml of suspension ($1.5 . 10^{8}$, $5.6 . 10^{8}$, $1.8 . 10^{8}$).
   CFU describes the colonies forming units; they grow on the surface of agar in separate colonies.

3. The method of direct prints on an agar plate with the surface 28 cm^2 was chosen to monitor the number of colonies before experiments and during experiments. The blood agar was chosen for experiments with both staphyloccocuses end enterococcuses. Function of selective agars were verified in pre-experiments. Because they did not work well, the results were not included in the tables in the conclusion.
   The direct prints were used because we use them during examining the washing, cleaning and disinfecting surfaces in the health facilities. They are simple and are minimally altered by conditions in a lab.

4. The paints with titanium oxide (TiO_2) were applied on the glass plates, 15 x 20 cm (300 cm^2) large with the rough surface. The control glass surfaces without the tested paint applied on them were the same size and the same surface texture.

5. For illumination the UVA light lamp Omnilux with the wave length 370 nm was used.

Based on the recommendation of manufacturer and accessible methodologies the period of illumination was set to 20, 60 and 120 minutes. The control measurements without illumination are marked as 0 minutes.

6. To monitor the long-term photocatalytic activity of TiO_2, all surfaces were controlled after 24 hours and after five days. The glass plates were stored in a closed laminar box and they were protected from light and humidity decrease by an aluminum foil.

7. The experiments were performed as follows:

   Enterococcus faecalis CCM 4224: the amount of 300 micro liters of suspensions with the density $1.5 . 10^8$ and $5.6 . 10^8$ were uniformly spread on the surface in both experiments, it means that the surface was covered by bacteria of volume 10 micro liters per 1 cm^2. After the direct print such amount of bacteria grew on the blood agar in amount marked as UA – uncountable amount. After 24 hours of growing on agar in thermostat at 36 +- 1°C the colonies were mutually interconnected, almost fused. If it was possible to count the colonies on the agar surface, the number up to 100 colonies was expressed in units, over 100 colonies it was rounded into tens of colonies. Three prints were performed during each collection from tested surfaces. In tables, for the clarity, there are the average numbers relating to one plate, this is 28 cm^2. If the amount of colonies was uncountable, it was expressed as UA or 3 x UA.
   The experiment with Staphylococcus aureus CCM 3953 suspension was performed equally.

Results

1. Table one shows the different survival of Enterococcus faecalis on plain surfaces and surfaces painted with TiO_2. The collections performed within the time interval from 0 to 120 minutes after the suspension dried were showing uncountable amount of surviving bacteria (K1). During the illumination, which took 0 – 120 minutes, TiO_2 caused the overall decrease of number of colonies from UA to 1893, 1667 and 767. The complete number of colonies was 4327 (K1 TiO_2). The collections performed after 24 hours showed another decrease down to 8,8 % of the original number of colonies.

2. During 24 hours the bacteria were dying in the spontaneous way. We must compare the effect of paint with TiO_2 itself with the number of countable colonies during the same period of time. From the whole amount of 1573 colonies the number decreased down to 379, which is 24 % of the compared amount after 24 hours elapsed.

3. Table two shows the results of the second experiment with Enterococcus faecalis of the higher density $5.6 . 10^8$. The collections performed within the time interval from 0 to 120 minutes after the suspension dried showed uncountable amount of surviving bacteria on all prints (K1).

4. The collections performed after 24 hours showed the overall decrease down to UA, UA, 297 colonies. During 24 hours the bacteria were dying in the spontaneous way.
   We must compare the effect of paint with TiO_2 itself with the number of countable colonies during the same period of time. From the whole amount UA, UA, 297 colonies the number decreased down to 462. It is not possible, however, to express the decrease in per cent of the compared amount. But it is obvious – decrease down to total 462 colonies.

5. The collections performed after five days showed the apparent influence of TiO_2 presence in the paint. During five days the bacteria were dying in the spontaneous way and their amount decreased down to 618 colonies. We must compare the effect of the paint with TiO_2 itself with the amount of countable colonies during the same period of time. The total amount of 618 colonies decreased down to 5 on surfaces with TiO_2. This means the decrease to 0.8 % of the compared amount after five days elapsed.

6. Table 3 shows the result of experiment with Staphylococcus aureus, density $1.8 . 10^8$. The collections performed in the time interval from 0 to 120 minutes after the suspension dried showed the uncountable amount of surviving bacteria on all prints (K1). During the illumination, which took 0 – 120 minutes, the amount of bacteria decreased from 3 x UA down to the countable average amount of 37 – 173 colonies. The total amount of colonies was 370.

7. The collections after 24 hours showed the overall decrease down to 985 colonies. During 24 hours the bacteria were dying in the spontaneous way. Therefore we will compare the effect of the paint with TiO_2 itself with the amount of countable colonies during the same period of time. The total amount of 985 colonies decreased down to 370. This means the decrease down to 37,6 % of the compared amount of surviving bacteria not affected by TiO_2.

8. The collections performed after five days showed the apparent influence of TiO_2 presence in the paint. The bacteria were dying in the spontaneous way and the number of colonies decreased down to 519. Therefore we will compare the effect of paint with TiO_2 itself with the amount of countable colonies during the same period of time. The total amount of 519 colonies decreased down to 7 on surfaces with TiO_2. This means the decrease down to 1.3 % of the compared amount after five days elapsed.

Discussion

The photocatalytic antimicrobial effect of titanium oxide is known both in a literature and a practice. Some exceedingly optimistic results in lab conditions do not prove its worth in a practice. The efficient substance in a paint has to fit the size of nanoparticles, this is 10^{-9}.

The results of individual experiments in the same lab can differ. As it follows from our results, it is necessary to choose the adequate density of bacteria.

The tribes, which survive in the long-run in the common lab conditions in a dried suspension are suitable for experiments. It follows from the experiments that the amount of viable bacteria significantly decreases. We have to take note of spontaneous exponential dying of bacteria and we must compare the results of physical and chemical influences with the control surface after the same period of time elapsed.

The finding, that the period of time, during which bacteria are exposed to TiO_2 nanoparticles matters, is very interesting but expected. The collections performed immediately after a plate with the suspension applied on its surface is illuminated, cannot be evaluated by the countable amount of bacteria if the density is too high. If we lower the original density, the amounts of bacteria on print plates are low and it is hard to compare them. Our experience shows, that the original population of bacteria must be higher, but it is suitable to perform the evaluation of

TiO_2 influence not before 24 hours. We can logically suppose, that each physical and chemical influence of examined substance requires the adequate exposition. It was not our goal to determine the necessary time of exposition.

Conclusion

The experiments with the paint containing TiO_2 showed the decrease of Enterococcus faecalis colonies down to 8.8 % of the original amount after 24 hours elapsed. When the high density population was used, the original uncountable amount decreased down to 462 colonies. In case of Staphylococcus aureus the decrease was 37.6 % of the compared amount of surviving bacteria without the influence of TiO_2.

Two experiments during which the collections were performed after five days show the decrease of enterococcus occurrence down to 0.8 % and the decrease of staphylococcus down to 1.3 % of the compared amount.

The experiments provide the proof of the strong antimicrobial effect of tested paint prepared by the company Ing. Jan Procházka, Advanced Materials – JTJ Inc., 273 01 Kamenné Žehrovice 23. I recommend applying and verifying the paint during field tests in health facilities.

RNDr. Erich Pazdziora, CSc
guarantee of tests

The protocol contains:
4 pages of text
2 pages of attachment

Table 1: Enterococcus faecalis, density $1.5 . 10^8$

Intervaly odběrů v minutách	K1 odběry v čase 0-120´	K24 odběry po 24 h	K1 TiO_2 v čase 0-120´	K24 TiO_2 po 24 h
0	3 x NM	97	NM	81
20	3 x NM	516	1893	147
60	3 x NM	690	1667	137
120	3 x NM	270	767	14
Celkem	3 x NM	1573	4327	379

K1 – Control plate without TiO_2 and without illumination – dried suspension taken from surfaces which were illuminated 0 – 120 minutes
K24 - Control plate without TiO_2 and without illumination – dried suspension taken after 24 hours
K1 TiO_2 – plate with TiO_2 coat which was illuminated - dried suspension taken from surfaces which were illuminated 0 – 120 minutes
K24 TiO_2 - plate with TiO_2 coat which was illuminated - dried suspension taken from surfaces, which were illuminated 0 – 120 minutes, after 24 hours elapsed

Table 2: Enterococcus faecalis, density $5.6 . 10^8$

Intervaly odběrů v minutách	K1 odběry v čase 0-120´	K24 odběry po 24 h	K1 TiO_2 v čase 0-120´	K24 TiO_2 po 24 h	K 5 v čase 0-120´	K5 TiO_2 po 5 dnech
0	3 x NM	NM NM 150	NM NM 285	138	158	5
20	3 x NM	NM NM 72	NM NM 280	187	154	0
60	3 x NM	NM NM 43	330	137	134	0
120	3 x NM	NM NM 32	208	43	172	0
Celkem	3 x NM	NM NM 297	NM NM 1103	462	618	5

K1 – Control plate without TiO_2 and without illumination – dried suspension taken from surfaces which were illuminated 0 – 120 minutes
K24 - Control plate without TiO_2 and without illumination – dried suspension taken after 24 hours
K1 TiO_2 – plate with TiO_2 coat which was illuminated - dried suspension taken from surfaces which were illuminated 0 – 120 minutes
K24 TiO_2 - plate with TiO_2 coat which was illuminated - dried suspension taken from surfaces, which were illuminated 0 – 120 minutes, after 24 hours elapsed
K5 – control plate without TiO_2 and without illumination - dried suspension taken from surfaces, which were illuminated 0 – 120 minutes, after 5days elapsed
K5 TiO_2 after 5 days - plate with TiO_2 coat which was illuminated - dried suspension taken from surfaces, which were illuminated 0 – 120 minutes, after 5days elapsed

Table 3: Staphylococcus aureus, density $1.8 . 10^8$

Intervaly odběrů v minutách	K1 odběry v čase 0-120´	K24 odběry po 24 h	K1 TiO_2 v čase 0-120´	K24 TiO_2 po 24 h	K 5 v čase 0-120´	K5 TiO_2 po 5 dnech
0	3 x NM	480	57	88	128	5
20	3 x NM	227	173	117	119	1
60	3 x NM	150	103	39	131	1
120	3 x NM	128	37	24	141	0
Celkem	3 x NM	985	370	268	519	7

K1 – Control plate without TiO_2 and without illumination – dried suspension taken from surfaces which were illuminated 0 – 120 minutes
K24 - Control plate without TiO_2 and without illumination – dried suspension taken after 24 hours
K1 TiO_2 – plate with TiO_2 coat which was illuminated - dried suspension taken from surfaces which were illuminated 0 – 120 minutes
K24 TiO_2 - plate with TiO_2 coat which was illuminated - dried suspension taken from surfaces, which were illuminated 0 – 120 minutes, after 24 hours elapsed
K5 – control plate without TiO_2 and without illumination - dried suspension taken from surfaces, which were illuminated 0 – 120 minutes, after 5days elapsed
K5 TiO_2 after 5 days - plate with TiO_2 coat which was illuminated - dried suspension taken from surfaces, which were illuminated 0 – 120 minutes, after 5days elapsed.

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