



EFFICACY OF ENVIRONMENTALLY BENIGN AND DURABLE TiO₂ NANOCOMPOSITE AS ANTIMICROBIAL COATING SOLUTION

Microbiology

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ABSTRACT

Since last many years, we are trying to improve our hygiene solutions using various traditional antimicrobial chemicals to make our environment clean but artificial, which created huge problems like damaging the ecosystem, generating antimicrobial resistant species etc. TiO₂ is a well-known inhibitory for its bactericidal effect. TiO₂ based nanocomposites subjected to light excitation are remarkably effective in eliciting microbial death. Antimicrobial efficacy of the nano TiO₂ is expected due to the photocatalytic action which is improved by modifying nano-environment. The TiO₂ nanocomposite coating has been applied on the glass plate. The antimicrobial action of the coating was investigated qualitatively and quantitatively against various unicellular life form. Good inhibition was observed with a nanocomposite coating on the quartz plate. The quantitative examination was estimated by survival ratio using CFU on nutrient-rich media. TiO₂ nanocomposite is working as a catalyst, not utilized during its action, which means it will provide durable protection. From this study, TiO₂ nanocomposite can be a better option over traditional solutions.

KEYWORDS

Photocatalytic, Nanotechnology, hygiene, Ecobactix®, TiO₂

INTRODUCTION

One of the major issues around cleaning or hygiene solution is microbes can be absorbed into the pores of porous or semi-porous solid surfaces, enables a significant hazardous pathway for humans (1). This issue of contamination by certain microorganisms in healthcare area may create a deadly condition, i.e. HAIs (Hospital Acquired Infection) (2). It has been estimated and predicted HAIs accounts 7-10% (3).

Generally, it is controlled by using chemical-based compounds which is effective but has many problems too (4). They are not long term in protection that is leaving a new room for new development.

TiO₂ nano particle was tested and reported with excellent antimicrobial activity in the efficient removal of such absorbed microbes (5, 6, 7). TiO₂ nano particle may not show a considerable antimicrobial activity in the form of metal oxide and metal salt alone, because they tended aggregation (8). The silver nano particle has excellent antimicrobial property. Silver nano particles are not a stable and quick release of ions may shorten its effectiveness (9). However, the stability and slow release of metal ions from nano particle are effective properties that can be tuned by appropriate synthesis. For this reason, new methods core-shell have been suggested (10).

One of the most important factors in antimicrobial activity is light for TiO₂ nano particle. TiO₂ nano particle becomes activated against pathogenic bacteria when exposed to ultraviolet (UV) light, photocatalytic action (11). For silver nano particle, various factors effects on the level of antimicrobial action. Silver shows antimicrobial action through oligodynamic action.

TiO₂ has been widely applied as a coating because of its high photo activity, non-toxicity and strong self-cleaning properties, but there are two main problems, activation only under UV light and recombination of generated electron-hole pair (11,12). To solve this problem, we have changed its nano-environment and resulted in nano composite is studied for its antimicrobial action.

EXPERIMENTAL

Materials

Quartz slide for TiO₂ coatings was purchased from Borosil®, India. Ecobactix® (Product of Framtix Holding AB, Sweden), nano-modified TiO₂ nano-coating, as used as TiO₂@Ag coating. Antimicrobial studies were performed using a bacterial strain *Escherichia coli* and *Staphylococcus aureus* fungal strain *Candida albicans*.

Antimicrobial experiments were performed using the Millipore MilliQ system. All microbiology media component was bought from Himedia, Mumbai.

Preparation of coating

Quartz slide was sonicated and cleaned with ethanol to remove all kind of dirt particles. Once the slide is dried, Ecobactix® was spread using high-pressure low volume sprayer, allowed it to dry at room temperature. Once the slide is dried, kept it for incubation for 48 hours.

Evaluation of the antimicrobial activity of coatings:

The antibacterial properties of coatings were determined using the Japanese Industrial Standard JIS Z 2801:2000 (13). *E. coli* was inoculated on Luria-Bertani (LB) broth and incubated at 37°C on a rotary shaker. *E. coli* suspension including 1×10^5 Colony Forming Units (CFU per ml) in phosphate buffer saline was prepared for antibacterial tests. The diluted *E. coli* inoculum was applied to the coated slide and uncoated quartz slide were analyzed as control samples. After the application of the bacterial suspension on the slide, each coating was covered with an aseptic quartz cover slip and incubated in the moist chamber at 37°C for 4 h. Next, the *E. coli* suspensions were washed and serial dilutions were prepared in the phosphate buffer saline. Diluted bacterial suspensions were spread on LB agar and incubated at 37°C for 24 h. Tests were performed under three different conditions, under UV light, under visible light and dark condition. The antibacterial potential of tested plates was calculated as a ratio of growth inhibition against control samples. All samples were tested in triplicate and individually analyzed.

In vitro Recontamination study

Similar step as per the evaluation of antimicrobial activity, the bacterial suspension has grown on LB broth and fungal culture on Sabrood Dextrose (SD) broth. Next, slides are treated with bacterial and fungal suspension frequently as mentioned in the table. It is done in a controlled environment and on repeated based (14). Details are shown in table 1.

RESULTS

The antimicrobial activity

The antibacterial potential (ABP) of the coated surface was calculated as a percentage of growth inhibition against control samples. The ABP is the number of live *E. coli* cells (CFU) on Coated surface to the uncoated sample under the same condition. From the observation of three sets, the number of viable bacteria decreased at least three-fold after the 4-hour UV exposure. Remarkably, 2 log AMA (antimicrobial action) was also achieved under visible light exposure. Interestingly, the nanocomposite coatings also had 1 log AMA even in the dark. Detailed results are shown in figure 1.

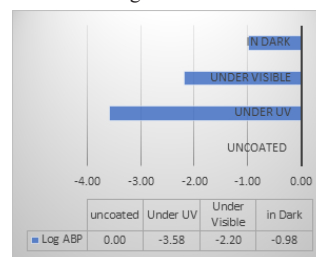


Figure 1: Antimicrobial action under various condition, semi-logarithmic plot: condition against the log of antibacterial potential ratio.

Recontamination study

This study was done under visible light. The effective reduction was observed which was showing the same result again, two-fold reduction. Detailed result is mentioned in table 1.

Table – 1 Revived Cfu/ml In Recontamination Study Initial Concentration Was 0.5×10^6 Cfu

Microbes	CFU mL ⁻¹ survived after 4 hours of visible light					
	Day 0	Day 2	Day 7	Day 14	Day 30	Day 50
<i>E. coli</i>	250 ± 52	278 ± 72	150 ± 84	300 ± 29	210 ± 56	378 ± 20
<i>S. aureus</i>	438 ± 80	340 ± 102	286 ± 56	440 ± 54	300 ± 86	356 ± 115
<i>C. albican</i>	110 ± 32	130 ± 19	80 ± 34	200 ± 31	98 ± 30	116 ± 20

DISCUSSION

Above result shows, EcobactiX® TiO₂ coatings for antimicrobial touch surfaces applications works excellent even under visible light activation and on recontamination. TiO₂ activates under UV light and generate electron and hole pair. This excitation radiation can be red-shifted by changing nano environment i.e doping (15, 16), composite with another particle (17), core-shell (18) etc. Ecobactix® has already changed the nano environment for optimum action. From chart 1, under visible light is reducing the growth of bacteria. Though it is red shifted still it is showing maximum reduction under UV light that maybe the action of UV light. Result of dark condition, because of the action of oligodynamic action. Photocatalytic action works only under light condition. This proves coating works well in the light as well as in dark condition.

From recontamination study, it is also clear that time is not affecting the action of the coating as it working as a catalytical action which kills bacteria (19). Catalyst doesn't take part in the reaction, so they will not be utilized during the reaction (20). The surface will remain active all the time for the long term until it is covered or removed.

It is not utilized so it is being reused again and again. And being active all the time on the surface, making live protecting surface. Continuously reducing microbes from the applied surface through a combination of photocatalytic and oligodynamic effects. These effects have multiple modes of action which reduce the chance of generating mutants (21). So, it will reduce loads of other antimicrobials on the coated surface and help in creating a sustainable environment too.

CONCLUSION

Modified nano-environment of TiO₂ makes Ecobactix® a good antimicrobial coat. It has a good action in light and dark condition. As it is working on catalytic action, the coating will not be utilized during the reaction. So, it will give long-term protection.

The differences in the level of *S. aureus* growth inhibition in all experiments for each sample were negligible, which indicates their great antibacterial stability and durability.

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