Investigation of Antibacterial Behavior of Polymethyl Methacrylat Supported TiO$_2$ Nanophotocatalyst Film

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Abstract: Polymethylmethacrylat/TiO$_2$ nanophotocatalyst is synthesized by irradiating the solution of TiO$_2$ in methylmethacrylate monomer by γ-ray. In this method, polymerization of the methylmethacrylate monomer and the TiO$_2$ reduction occurred simultaneously. The structural characterizations of the polymethylmethacrylat / TiO$_2$ nanophotocatalyst are determined by FTIR spectroscopy and SEM measurements. The SEM image shows that the TiO$_2$ nanoparticles disperse in the polymethylmethacrylat matrix with a relatively uniform distribution. The antibacterial studies show that the polymethylmethacrylat / TiO$_2$ nanophotocatalyst is antibacterial against E.coli, as a model for gram-negative bacteria.

Keywords: TiO$_2$, PMMA, Film, Antibacterial activity

1. Introduction

Titanium dioxide (TiO$_2$) as a photocatalytic antibacterial material has considerable beneficial properties such as incompatibility, high potential for self-cleaning and high antibacterial activity and is now the basis for a number of commercial antibacterial products [1,2]. Not surprisingly, there is an increasing interest in combining photocatalytic activity of TiO$_2$ and hydrophobic polymer for preparation of polymer supported TiO$_2$ [3-5]. So, polymeric materials are a good candidate to use in the health care industry and environment materials [6,7]. Mills et al. produced low density polyethylene (LDPE)–TiO$_2$ films by an extrusion method and tested photocatalytic antibacterial activity, via the destruction of Klebsiella. Polymeric material such as Poly Methyl Methacrylate (PMMA) due to hydrophobic nature and low surface energy leads to low bacterial adhesion [2]. The photocatalysis and ultraviolet or visible absorption properties of TiO$_2$ nanoparticles (NPs) that can also be utilized in antibacterial finishing and anti-ultraviolet finishing of textiles to meet the demand of multi-functional finishing of textiles [8-10].

The aim of this examination is using of polymethylmethacrylat/TiO$_2$ (hereafter PMMA/TiO$_2$) NPs that was prepared by ionic liquid based microemulsion in continuation of our research works on microemulsion systems as soft template for nanomaterial’s synthesis [11,12]. In previous report, at the first time they are prepared and characterized the PMMA/TiO$_2$/IL as highly efficient visible light photocatalyst and as an application in waste water treatment field the photodegradation of methylene blue dye was investigated [13]. Having this in mind, in current work, as a new application the antibacterial effect of PMMA/TiO$_2$ nanocomposite based on the destruction of clinical strain of Gram-negative Klebsiella spp. (MTCC 7407) with, various loading of TiO$_2$ was tested in harvest of visible light. With the above in mind, we hope to offer practical approach with our suggested work for a direct industries application because TiO$_2$ conveniently available in market and the fabrication method is easy.

2. Methodology

2.1. Materials

Hydrophilic ionic liquid, ([BMIM][BF$_4$]) and the nonionic surfactant Triton X-100 were purchased from Sigma- Aldrich. The TiO$_2$ NPs used in this study was Degussa P25 (ca. 80% anatase, 20% rutile, with a BET...
surface area of 50 m²/g and particle size of less than 15 nm). Methyl methacrylate (MMA) monomer (AR grade), benzoyl peroxide (BPO) and 1-butanol was received from Merck. All aqueous solutions were prepared with deionized water.

2.2. Preparation of the modified PMMA/TiO₂

In making the PMMA/TiO₂ NPs microemulsion consisting of hydrophilic ionic liquid [BMIM][BF₄] (3 wt.%), MMA (88.94 wt.%), TX-100 (5 wt.%) and 1-butanol (2.24 wt.%) with different loading of TiO₂ NPs was prepared at 60°C. Benzoyl peroxide (BPO) as an initiator (0.2 wt.%) based on the weight of MMA was added to a range of variety formulated of TiO₂-microemulsion for starting polymerization process. Finally some photocatalyst-embedded PMMA films were prepared and for labeling purposes, the resultant transparent nanohybrid thin films of TiO₂ in PMMA were termed as S1, S2, S3, and S4 referring to the amount (wt.%) of TiO₂ corresponding to 0.008 wt.% PMMA/TiO₂, 0.01 wt.% PMMA/TiO₂, 0.012 wt.% PMMA/TiO₂ and 0.014 wt.% PMMA/TiO₂ respectively. No apparent visible phase separation was observed during polymerization process for all samples. Pure PMMA was also prepared under otherwise the same condition and identically formulated microemulsion without TiO₂ loading. All the films produced were of the uniform thickness with an average central thickness as measured using a micrometer, was 100 μm. All the films were optical transparent, as illustrated by the photograph of example applied PMMA/TiO₂ in Figure 1, indicating transparency and flexibility of films.

2.3. Pilot study

A pilot study was performed to assess the antimicrobial activity of 3 wt.% and 5 wt.% of TiO₂ in PMMA in three forms, using methodology described below. Since comparable antimicrobial activity against S. aureus was seen in both concentrations, 3 wt.% of NPs was considered, for economic reasons to the end user and less effect on the mechanical properties of denture base resins.

2.4. Preparation of samples with TiO₂

Sample preparation of TiO₂-PMMA composite was done by 3 methods. Mould spaces for specimens were prepared using wax pieces (5 mm™ 5 mm™ 2 mm thickness) in a dental flask. 3 wt.% and 5 wt.% TiO₂ by weight of polymer i.e. 0.3 g and 0.5 g of NPs were weighed and mixed with 1.7 ml of methyl methacrylate monomer and then 8.55 g of polymer (DPI Heat Cure, India) was added to the same. It was thoroughly mixed and on reaching the dough stage was heat cured.

3. Results

3.1. Antibacterial activity results

The zone of inhibition toward clinical isolate Klebsiella spp. with pure PMMA and PMMA/TiO₂ NPs with different loading of TiO₂ was evaluated under visible light. The data shows that PMMA/TiO₂ NPs exhibited the antibacterial activity against Klebsiella spp. bacteria than pure PMMA. With increasing TiO₂ dosage, firstly antibacterial activity increases and with further loading, the antibacterial activity decreases. The maximum antibacterial efficiency was belonging to sample S2 of PMMA/TiO₂ NPs and has broad spectrum.

The determined zones of inhibition with pure PMMA as a control sample, S1, S2, S3 and S4 samples were 0, 8 ± 0.50, 20 ± 0.72, 19 ± 0.51 and 12 ± 0.28, respectively. Figure 6, presents the zone of inhibition against toward Klebsiella spp. as an example of Kirby-bauer method. As expected, the antibacterial activity was found to increase with the increasing dosage of TiO₂ and loss of antibacterial activity can be observed at high loading of TiO₂ due to aggregation of NPs in polymer matrix which causing a decrease in the number of surface active site. The main killing mechanism for the antimicrobial effect of TiO₂ photocatalysis is attributed to the hydroxyl radicals and oxygen reactive species as part of the photocatalytic mechanism. The creation of electron–hole pairs during visible light irradiation, which, in turn, can lead to the photocatalytic process. The hydroxyl radicals and reactive oxygen species in which the bacterial cell membrane is the primary oxygen reactive species attack side, leading to lipid peroxidation cell membrane. Youssef et al. reported the killing mechanism of the microorganisms originally may be explained as follows: the degradation of the cell wall and cytoplasmic membrane by hydroxyl radicals and hydrogen peroxide that initially leads to leakage of cellular contents. Finally cell lysis followed by complete mineralization of the organism. As can be seen from Figure 2, no zone of inhibition was observed for PMMA in contrast of the conductive polymer such as polypyrrole. Key factors such as electrostatic adsorption between conductive polymer and bacteria, higher molecular weight, surface hydrophilicity and...
direct contact between polymer and bacteria cell lead to existence of antibacterial activity of pure conductive polymer.

4. Conclusion
The possible physicochemical interaction between the TiO$_2$ nanophotocatalyst and PMMA was tested by FT-IR. It is known that the shift of the peaks toward the lower wave numbers in the FT-IR spectrum is an indicator of the chemical bonding of surfactant onto the nanoparticle surface. The spectra of the neat PMMA and the PMMA/TiO$_2$ composite are shown in Figure 1. As can be seen all the characteristic absorption peaks of standard PMMA including: the sharp absorption at 1728 cm$^{-1}$ (C=O groups), the peaks at 1147 and 1193 cm$^{-1}$ (C–H deformations), and 1244 and 1272 cm$^{-1}$ peaks (C–C–O stretch coupled with C–O stretch), are observed in neat and PMMA stabilized Ag nanoparticles indicating the occurrence of MMA polymerization by $\gamma$-ray.

**Fig2.** FT-IR spectrum of the a) neat PMMA b) PMMA/TiO$_2$ nanophotocatalyst.

UV-Vis absorption spectra are quite sensitive to the formation of Ag NPs because of the position of the surface plasmon absorption peak depends on their particle diameters and shapes. Therefore UV-Vis absorption spectra of the neat PMMA and PMMA/TiO$_2$ were obtained (Figure 3). In comparison to neat PMMA, the PMMA/TiO$_2$ composite shows an absorption band at about 428 nm, which is consistent with the established absorbance wavelength of TiO$_2$ nanophotocatalyst to determine the size and distribution of the TiO$_2$ nanophotocatalyst, scanning electron microscopy was performed. The SEM micrograph of PMMA/TiO$_2$ composite indicates that Ag particles with mean size of 90±14.4 nm disperse in the PMMA matrix with a relatively uniform distribution. This confirms that when reduction of silver ions and the polymerization of monomer take place simultaneously during irradiation, the TiO$_2$ nanophotocatalyst are homogeneously dispersed in the polymer matrix.

**Fig3.** UV-Vis absorption spectra of neat PMMA and PMMA/TiO$_2$ nanocomposite.

Literature reveals many investigations wherein antifungal & antiseptic agents were incorporated/coated on denture base resins or soft liners. However their effect was not persistent, as these agents were rapidly released. Hence, the main objective of this study was to develop and evaluate antibacterial activity of a denture base resin with minimum percentage of visible light photocatalytic additive. Various techniques of preparing visible light activated TiO$_2$ have been proposed including sol-gel technique, N doped, C doped, Co ion doped – TiO$_2$ photocatalyst. The disadvantages of doped materials were low surface energy because of using high temperature, requirement of expensive chemicals and synthesis methods and an increase in carrier recontamination. Randron et al. recommended the use of TiN as an alternate precursor in peroxide based route because of its air and moisture stability, organic and chloride ion free route, simplicity and low cost. In concurrence with the above study, authors would like to state here that TiO$_2$ NPs were synthesised by a simple and organic free precipitate technique using TiN as a precursor. patterns of the as-prepared & anatase phase confirmed the respective phases of TiO$_2$. The particle size of TiO$_2$ as calculated by Debye-Scherer equation was 31 nm. The obtained phase transformation from yellow amorphous to anatase at 800 °C and particle size were consistent with the previous work. Since the anatase form of TiO$_2$ showed photocatalytic phase which was the required form in our work, we continued the use of anatase form in our work.
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Fig 4. Representative SEM photomicrograph of surface of poly methyl methacrylate incorporated with TiO2 NPs.

Fig 5. Number of E. coli colonies grown on control plates without PMMA/TiO2

5. References
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