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Study of Bacterial Cell Colonization on Plasma Induced Bio-Adoptable Polymer Nanocomposites Membranes

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Summary: Polymers are receiving great interest for synthesis of biomedical devices but to use them for any biomedical application they must show biocompatibility. In this work we have enhanced bio-compatibility of Poly (methyl methacrylate) (PMMA) using TiO_2 nanoparticles (NPs) and plasma irradiation. Spherical shape, 15–20 nm TiO_2 NPs were synthesized using wet chemical method and used to prepare polymer nano-composite (PNC) membranes of 20 micron via solution casting method. Further these membranes were irradiated by low temperature glow discharge Oxygen (O_2) ion plasma. Surface modifications produced by nanoparticle mixing and plasma irradiation were characterized by Optical Microscope, Scanning Electron Microscope (SEM) and Atomic Force Microscope (AFM). Results show increase in surface roughness and porosity thus changes surface energy and surface reactivity that helps to enhance selective bacterial growth on surface of the polymer nanocomposite membranes. The results are described in the paper.

Keywords: biological response; O_2 ion plasma; polymer nano-composite (PNC); surface modification; TiO_2 nanoparticles

Introduction

Polymers have became very important materials in modern manufacturing processes. They offers wide variety of chemical,[1] electrical[2] and mechanical[3] properties which are applicable in numerous applications like architecture,[4] automotive,[5] construction,[6] design,[7] electronics,[8] energy,[9] furniture,[10] lighting,[11] medical,[12] optical fiber communications[13] and sanitation.[14]

Polymer nanocomposites, in principle, can be formed from clays and organoclays in number of ways including in situ polymerization,[15] solution and latex methods.[16] Numerous experimental investigations on polymer nanocomposite (PNC) have indicated that these materials exhibit new and sometimes improved properties that are not displayed by individual phases or their conventional composite counterparts.[17] They have many diverse applications in catalysis,[18] compatibility,[19] composite reinforcement,[20] cosmetic applications,[21] dye-sensitized solar cells,[22] self-assembled polymer films[23] and many other biomedical applications,[24,25] but permanent bonding, coating, printing etc. are difficult on many polymers without surface pre-treatment.

After surface treatment polymers can have significant benefits in many specific requirements/applications.[26,27] Physical and chemical surface modifications of polymeric materials without alteration of bulk properties are of great interest in many applications.[28–30] From last few decades polymers are also receiving pronounced awareness for fabrication of

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advanced materials used for biomedical applications.[31] Any substance or combination of substances, other than a drug, synthetic or natural in origin, can be used for any period of time, as whole or part of biological system, which treats, augments or replaces any tissue, organ or function of the system is called biomaterial.[32,33] These biomaterials are nonviable materials used in medical devices, intended to interact with biological systems.[34] Here it must be noted that the synthesized materials are foreign materials to biological systems and normally biological bodies do not accept foreign materials very easily till they shows superior biological response.[35,36] Biomaterials in contact with biological body have optimal combination of mechanical and surface characteristics so that they results superior performance in biological environments.[37,38]

No polymer, in general, possess surface-interface and chemical properties needed for biomedical applications like low surface energy, hydrophilicity, surface morphology, chemical functionality, high porosity and self-colonization ability of cells.[39,40] Hence researchers are working in the field of development/enhancement of these properties in polymers. Recently we have observed that apt amount mixing of nanoparticles into the polymer matrix can influence porosity of polymer membranes. Similarly complex nature of plasma due to presence of ions, neutrals and radiation in discharge makes low-temperature plasma widely useful in growing number of materials' fabrication/modulation processes including etching of complex patterns and surface modifications required for many biomedical applications.[41,42]

Plasma surface treatment usually refers to a plasma reaction that either results in modification of molecular structure of surface or atomic substitution.[43] Accelerated ions from plasma have sufficient energy to induce cleavage of chemical bonds on membrane surface and form macromolecule radicals that can subsequently initiate graft copolymerization[44] and etching of polymer depending on ion energy.[45] Various plasma components such as electrons, ions, radicals etc. are involved in these processes. Plasma treatment of polymer surface causes not only modification during plasma exposure, but also leaves active sites on surfaces which are susceptible to post-reaction.[45]

Hence, in this present work TiO_2-PMMA nanocomposite membranes were synthesized using solution casting method and irradiated with glow-discharge Oxygen (O_2) ion plasma to increase the porosity and cell-colonization ability of polymer membranes. The modified PNC were characterized by SEM and AFM for surface modification. Enhancement of porosity was examined by bacterial cell-colonization/selective bacterial growth.

Experimental Section

TiO_2 NPs were synthesized using $TiCl_3$ as described by Agrawal et al.[28] To obtain small and narrow particle size distribution of TiO_2 NPs, all the optimized parameters like reaction time, PH, synthesis temperature, concentration of Ti precursor and other experimental conditions were kept constant. $TiCl_3$ [99.5% pure] was cooled at $-20\,^{\circ}C$ in deep freezer, then 5 ml of this solution was taken into a capped stopping funnel and added drop wise into 300 ml of 25% NH_4OH solution at reaction temperature of $0\pm0.2\,^{\circ}C$, under vigorous stirring. Synthesis was done using 1:60 {$TiCl_3$:H_2O} volume ratio with a resulting TiO_2 NPs concentration of $\approx 12.5\,gL^{-1}$. To avoid aggregation of particles, pH of the solution was maintained at 2 using pH meter. This was achieved by slow mixing rate (3–ml/hr) of $TiCl_3$ into NH_4OH, so that the excess HCl gas can be evacuated and pH of the solution remained constant at 2. Reaction mixtures were made to undergo vigorous stirring for 24 hrs on magnetic stirrer at $0\,^{\circ}C$ for complete removal of HCl gas and to obtain white colored uniformly dispersed TiO_2 nanoparticle suspension.

Poly (methyl methacrylate) (PMMA) granules were used to prepare flat sheet

membranes. They were obtained as commercial grade from Loxim Polymers, Jaipur. Dichloromethane of extra pure grade was used as a solvent for preparing 10% polymer solution. Solution-casting method was used for preparation of Poly (methyl methacrylate) membranes. Poly (methyl methacrylate) granules were weighed and dissolved in dichloromethane (CH_2Cl_2) to prepare 10% solution. Solution was stirred on magnetic stirrer to ensure uniform dissolution and to enhance the rate of dissolution. This process was carried out at room temperature for around 3 hrs till a clear solution was formed that was then poured into flat-bottomed petri-dishes floating on mercury to ensure uniform thickness (20 ± 1 micron) of membranes. Solvent was allowed to evaporate slowly over a period of 10–12 hrs.

TiO_2 nanocomposite Poly (methyl methacrylate) membranes with doping concentration of 4% were prepared by solution casting method. Poly (methyl methacrylate) solution was prepared in the same way as mentioned above for pristine membranes. TiO_2 nanoparticles were dispersed in dichloromethane using ultra-sonicator. This dispersed solution was then added to Poly (methyl methacrylate) solution and stirred for around 30 minutes and the solution obtained was poured into flat-bottomed petri-dishes floating on mercury to ensure a uniform thickness (20 ± 1 micron) of nanocomposite membranes. Solvent was allowed to evaporate slowly over a period of 10–12 hours. The films so obtained were peeled off using forceps. Thickness of membranes was determined using ellipsometer, at 10–12 spots on each membrane.

Plasma irradiation set up at Department of Physics, University of Rajasthan consists of a source chamber with complete power supply and is connected to a vacuum system. The chamber was evacuated to a base pressure of 10^{-10} torr and the working pressure was maintained at 10^{-7}–torr by admitting oxygen gas. Oxygen (O_2) gas used for creating plasma is admitted into the source chamber using a flow controller. High voltage DC field (4 kV) was applied between two electrodes to generate DC glow discharge oxygen plasma. Magnetic field was used to confine the plasma in chamber. The plasma created was almost homogenous at low-pressure glow discharge. Energetic species in this plasma included oxygen ions, radicals, electrons and meta-stable photons in short-wave UV range.

LABOMED microscope was used for recording optical images. Images were stored in computer through CCD camera which was attached to the computer with standard software (Pixel View). Surface topography and roughness analysis before and after plasma-irradiation was carried using Carl ZEISS EVOR -18 scanning electron microscope (SEM) instrument operated at 15 kV. Surface topography was also recorded using Nanosurf Easyscan-2 atomic force microscope (AFM). For bacterial cell colonization study, the gram positive bacteria *E. coli* strain was obtained from Department of Botany, University of Rajasthan. The strain was then cultured in Luria Bertani broth (LB broth) and stock was prepared. To pick single cell colony or single cell colony culture to attain 100 CFU serial dilution of stock is performed in sterilized water.[46]

Results and Discussion

Size and shape of TiO_2 NPs may play an important role in controlling the porosity of nanocomposite membranes; hence here synthesized TiO_2 NPs were characterized by TEM. The TiO_2 NPs were vacuum dried and re-dispersed in acetone by ultrasonication, to ensure that there is no HCl present in the samples.[47] Figure 1 shows TEM image of TiO_2 NPs synthesized having spherical shape and particle size ranging from 15–20 nm. Individual particles in TEM images can be identified easily this shows no aggregations of TiO_2 NPs particles.

Biological response of most of biomaterials depends on their surface and

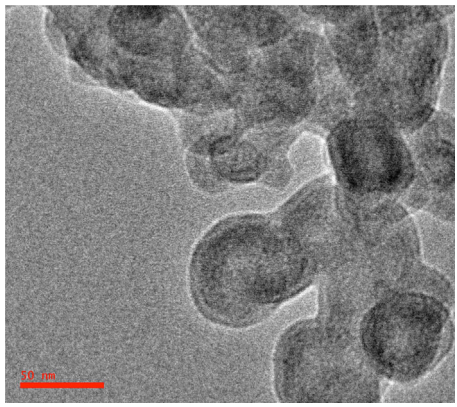

Figure 1.
Transmission electron micrographs (TEM) of TiO_2 NPs synthesized, having spherical shape and particle size ranging from 15–20 nm.

interface properties as this can significantly modulate cell/tissue adhesion, porosity etc. of biomaterials.[48,49] So identification of surface characteristics becomes very important. Here surface properties of membranes before and after plasma treatment has been identified by optical microscope, scanning electron microscope and atomic force microscope.

Optical micrographs show that pristine membrane has a very smooth surface but the plasma treatment increases its roughness. Nanocomposite membranes have comparatively high porosity but plasma treatment further enhances its surface roughness (Figure 2). For detailed investigation of surface modification SEM analysis was also done at higher magnifications. SEM images of polymer nanocomposite membranes before and after plasma irradiation are shown in Figure 3. Here also it is found that the porosity and roughness of the plasma treated membranes have increased which in turn increases the reactivity and adhesive properties of the membranes. AFM provides the best view of the surface

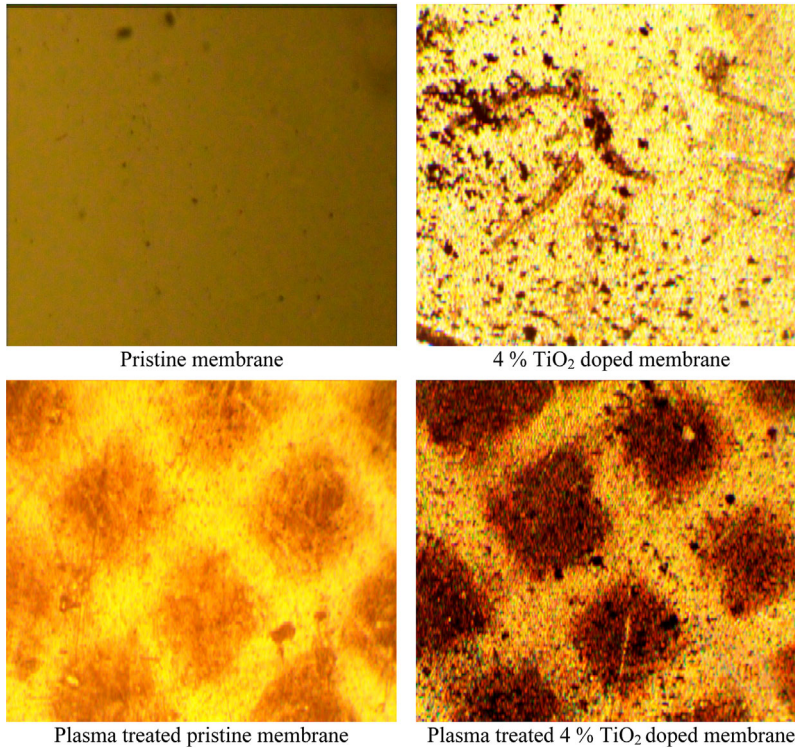

Figure 2.
Optical micrographs of pristine and TiO_2 doped membranes before and after plasma irradiation.

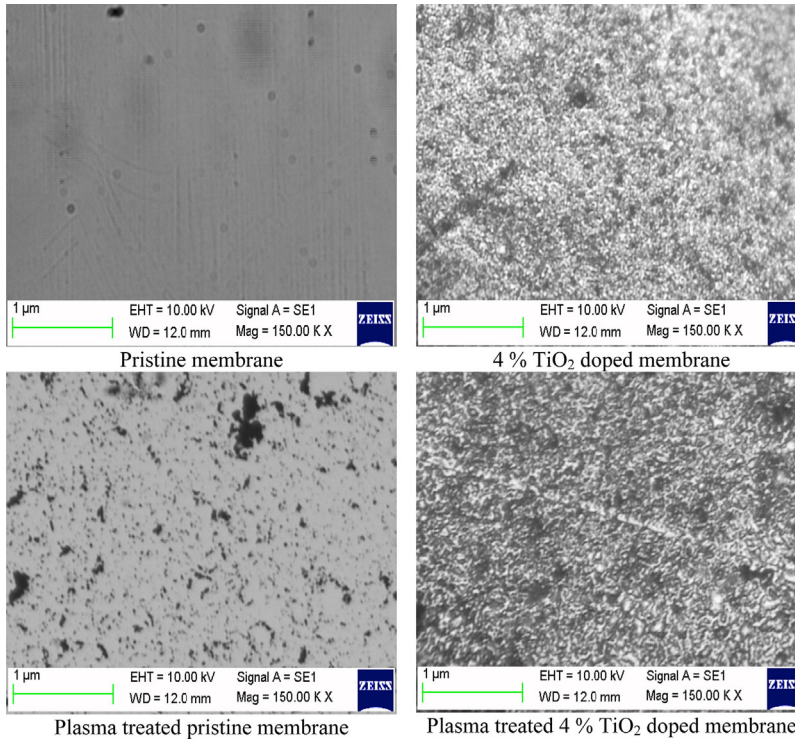

Figure 3.
SEM images of pristine and TiO_2 doped membranes before and after plasma irradiation.

morphology and topography in 3D, hence surface modification is also determined using AFM. Surface morphology of pristine and polymer nanocomposite membranes before and after plasma irradiation are shown in Figure 4. Both loading of nanoparticles in polymer and Oxygen plasma activation lead to increase in surface roughness of polymer membranes, but the granular structure of the polymer nanocomposite membranes is more pronounced after plasma irradiation. Analysis of the irradiated and un-irradiated surface morphologies show that plasma surface activation is more pronounced in polymer nanocomposite membrane as compared to pristine polymer membranes. As a result of plasma irradiation of polymer nanocomposite membrane, its surface roughness dramatically increases and sharp fragments appear on the modified surface. Results show plasma treatment produces surface and physical processing by atoms and ions on polymer surfaces, as described by Cui et al.[50] This increase in porosity of membrane is responsible for larger inter-fiber production, permeability and cell attachment. Changes in surface characteristics are related to plasma processing and effect induced by it on polymer surface (crosslinking, sputtering, surface cleaning, ion diffusion etc.) as observed in SEM analysis. Increase in surface roughness can also be explained by abrasion mechanism as explained by Dandia et al.[51] At start of plasma irradiation some abrasion among small peaks occurs but it results in increase in maximum peak height, for further processing of polymers lead to generation of localized abrasion that gives large number of small peaks as shown in Figure 4. Similar increase in surface roughness was obtained in PLLA after Ar ion plasma treatment by Paul et al.[10]

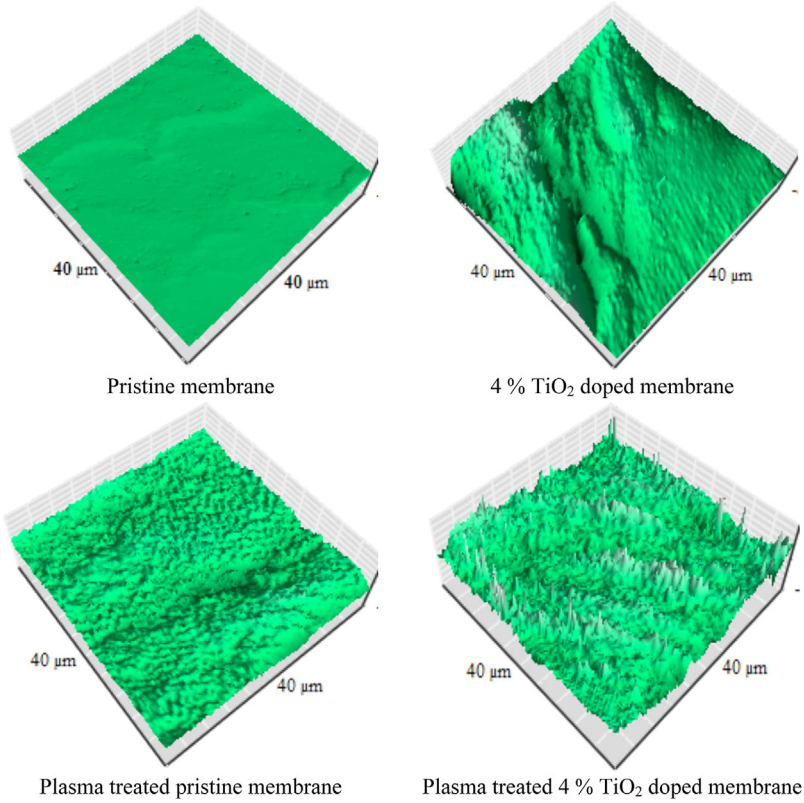

Figure 4.
AFM images of pristine and TiO_2 doped membranes before and after plasma irradiation.

Modulation of porosity of membranes can be identified by selective bacterial growth. Highly porous membranes are required for fabrication of artificial skin and they can be considered bio-adoptable as shown by Vijay et al. (2006).[14] Here also bio-adoptability of membranes was identified by selective bacterial growth, for this *E. coli* bacteria were chosen because it is relatively easy to handle, easily available and shows clear colonies within 24 hrs. Autoclaved membrane was mounted on food media (Autoclaved liquid nutrient Agar) and then few drops of bacteria solution (having 100 cfu/ml *E. coli*) were spread on irradiated and un-irradiated membranes by streaking and spreading method. These membranes were kept at 37° C in incubation chamber for 48 hrs. After incubation, the membranes were studied under optical microscope. In this study, the polymer membranes were working as a separating medium between bacteria and food media. Un-irradiated pristine polymer film was non-porous; it was acting as a passive layer between base *E-coli* and food media, hence no growth of bacteria was found on the surface of pristine polymer membranes. TiO_2 casted membranes showed little growth of bacteria on their surface due to some porosity present in these membranes, but the plasma irradiated polymer membranes shown enhanced bacterial growth (Figure 5). This can be attributed to the fact that membranes were becoming porous after plasma irradiation that allows food media to reach up to the bacteria to enhance their growth.

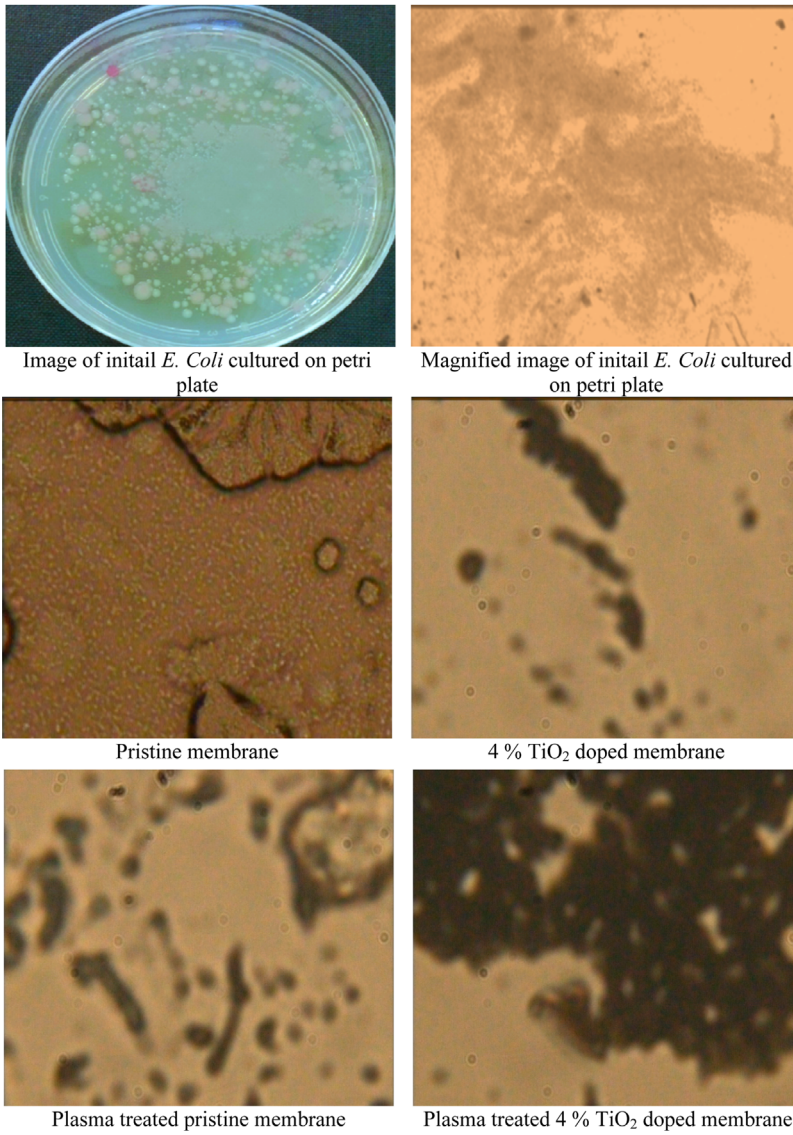

Figure 5.
Optical microscope images of selective bacterial growth on polymer membranes before and after plasma irradiation.

Conclusion

Pristine PMMA and 4% TiO_2 NPs casted PMMA membranes were prepared by solution casting method. These membranes were subjected to surface modification using Oxygen ion glow discharge plasma. Plasma surface modification technique applied here is an effective and economical surface treatment technique, drawing great interest in biomedical engineering; it has shown considerable improvement in surface morphology of polymer membrane as observed in SEM and AFM images. Enhancement in porosity was also observed by selective bacterial growth/cell colonization.

It must be remarkably noted that plasma irradiation significantly improved porosity, adhesion properties and surface energy of polymer nanocomposite membrane. This is a very important result, since low porosity of normal polymers is main drawback for their use in development of tissue engineering applications, as it gives poor cell penetration. Increase in porosity and reactivity that has been achieved here allows cell penetration for natural cell membrane applications.

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