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Supporting Information

Surface Modification of Medical Grade Biomaterials by Using Low-Temperature -Processed Dual Functional Ag-TiO_2 coating for preventing Biofilm Formation

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Experimental section

Biocompatibility study of Ag-TiO_2 coated medical device employing CAM

Ensuring the biocompatibility of medical devices is of utmost consideration for patient's safety. We studied the biocompatibility of Ag-TiO_2 using chick CAM (chorioallantoic membrane) assay. CAM is an extraembryonic layer which is formed during the avian development that function in gaseous exchange.[21] It acts as a temporary host for tissue samples, organs, and grafted materials so that the angiogenic action, as well as their viability and biological compatibility, can be investigated.[22] We performed CAM assay to study toxic effects of Ag-TiO_2 coated samples. First, we obtained the fertilized eggs from a poultry farm near IIT (BHU), Varanasi and placed them in an egg incubator for next 4 days maintaining temperature of 37°C. On the fifth day a small opening at the outermost shell was created gently with the help of forceps and then Ag-TiO_2 coated and uncoated coverslip was carefully placed on the developing chicken egg. The opening was then closed using parafilm. The eggs were then allowed to incubate within an egg incubator for 6 hours. A Stereo microscope (Magnus MagZoom TZM6 Trinocular Stereo Zoom Microscope) was used to capture images at 0 and 6 hours change in to access shift in blood vessel development. The images were then processed

by ImageJ and Angiotool software. The fold changes in length, size, junctions and endpoints of blood vessels images were examined using the images captured between 0 and 6 hours in response to both coated and uncoated sample coverslip.

Antibacterial efficacy of Ag-TiO_2 coated medical device in the presence of *E. coli.*

Initially, antibiotics, Ag NPs, and Ag-TiO_2 coated coverslips were kept in different wells of a 12 well plate, allowing incubation for 24 hours with GFP *E. coli* in luria broth (LB) media. Uncoated coverslips were kept as control. On the next day, 200 µl of LB media was collected from each well, transferred to a black 96 well plate and their fluorescence intensity was monitored. Both the coated and uncoated samples were washed gently using PBS solution. 100 µl of PBS was collected from each well and plated to access the number of colonies formed.

Anti-infection study of Ag-TiO_2 coated in *in vivo* chicken egg model.

One day old, fertilized chicken eggs were collected from a hatchery and incubated for a duration of four days. The next day, a small window was created on the top layer of the shell. 50 µl of *E. coli* (with an optical density of 0.6) were introduced to each egg to develop an infection model. These infected models were then subjected to treatment with Ag-TiO_2 coated and uncoated coverslip. In order to monitor the growth of blood vessels, microscopic pictures were taken at 0 and 2 hours utilizing a stereomicroscope. A pipette was employed to collect 10 µl of allantoic liquid after 4 hours. To determine the presence of infection, this fluid was diluted five folds with sterilized milli-Q water. 200 µl of diluted fluid was used for agar plating following incubation for a full day at 37 °C. On the next day, images of plates were captured using the Gel Doc imaging system.

Biofilm deposition

Ag-TiO_2 coated and uncoated medical devices were placed on different wells of a 12 well plate. Subsequently 2 ml LB media was poured on each well followed by 2 µl of freshly cultured *E. coli* (OD approx. 0.6). After that, the plate was left to incubate for 2 days at 37°C to allow biofilm formation.

Evaluation of antibiofilm effect of Ag-TiO_2 coated medical devices using Crystal violet (CV) staining

CV staining is an easy, straightforward assay widely used to access biofilm attachment over a substrate. *CV* (purchased from Sisco Research Laboratories Pvt. Ltd.) is basically a navy blue colored cationic dye that specifically stains the DNA and proteins within the EPS matrix making the entire biofilm visible and measurable. Initially, biofilm was allowed to grow on different Ag-TiO_2 coated medical devices keeping uncoated coverslip as control. After incubating for two days, the LB media was discarded, and the medical devices were cleaned 2 times utilizing PBS, dried, and then 400 µl of *CV* stains (0.1%) was applied. The devices were then placed on a rocker for 40 minutes.[23] The devices were again rinsed twice with PBS to remove unbound dye and dried for three hours. To liberate the bound *CV* dye from the biofilm, the devices were destain using 500 µl of a destaining solution (20% acetone + 80% ethanol) at room temperature for 20 minutes. After that, 200 µl of the resulting solution was placed on a 96-well plate and quantified at 595 nm absorbance using a microplate reader (Synergy H1-Microplate reader).[24, 25] Further analysis involves calculation Percentage biofilm inhibition in $AgTiO_2$ coated medical devices respect to uncoated control applying the given formula:

$$\text{biofilm inhibition (\%)} = \left(\frac{Ac - As}{Ac}\right) \times 100$$

Where, Ac denotes the absorbance of the control, and As denotes the absorbance of the sample at 595 nm.

Confocal Microscopy

We employed Confocal microscopy to check the attachment of bacteria over the coated and uncoated sample. *DAPI (4',6-diamidino-2-phenylindole)* and *PI (Propidium Iodide)* stains obtained from Sisco Research Laboratories Pvt. Ltd. were used to visualize the attachment of live and dead cells. In the beginning, the Ag-TiO_2 coated and uncoated coverslip was incubated with GFP-*E. coli* in LB media for 48 hours allowing biofilm formation. After 2 days, the coverslips were isolated from the bacterial suspension and rinsed twice with PBS. The coverslips were then placed on a clean slide. 200 µl of *DAPI* (15 µg/ml) solutions was added to the coverslips to visualize the attachment of both live and dead bacteria. The coverslips were washed utilizing PBS to eliminate unbound stain.

200 µl *PI* (10 µg/ml) was then added to the coverslip to stain the dead bacterial cells. The coverslips were again washed with the help of PBS to get rid of unbound dye. Next, 2.5% glutaraldehyde (obtained from Sisco Research Laboratories Pvt. Ltd.) was applied to fix the Ag-TiO_2 coated and uncoated coverslip to a glass slide. It was left to dry for half an hour in the dark. Thereafter, images were snapped employing Zeiss 510 Meta confocal microscopy system. ImageJ software was applied to count the total number of cells attached to the substrate.

Coating stability study

The stability of Ag-TiO_2 coating over medical devices was examined using various characterization techniques which includes UV visible spectroscopy, XRD and ICP MS. The absorbance of Ag-TiO_2 coated coverslips was recorded after 24 hours of coating and again absorbance was measured after a week to check the difference in absorbance spectra. Ag-TiO_2 coated coverslips were kept in different wells of a 12 well plate. To each well 1 ml of 10% Fetal Bovine Serum (obtained from Sigma-Aldrich) solution was added. The coverslips were incubated for 7 days at room temperature. The coverslips were then removed at specific time periods of 1,3,5 and 7 days. The removed coverslips underwent XRD to assess any changes in the material structure at each time point.

Ag^+ and Ti^{4+} release kinetics

Ag-TiO_2 coated coverslips were placed in different wells of a 6-well plate. We kept separate samples for separate study time points (1, 3, 5, and 7 days). 1 ml Fetal Bovine Serum (10% FBS) solution was added to each well and incubated for specific time points (1, 3, 5, and 7 days). The serum was collected after these specific time points from each group to study the release kinetics of silver (Ag) and titanium (Ti) from the Ag-TiO_2 coating into the surrounding environment. The FBS serum was collected from the 1st well after 1 day, from the 2nd well after 3 days, from the 3rd well after 5 days, and from the 4th well after 7 days to calculate the cumulative release profile of silver and titanium. 250 ml of 10% FBS solution obtained at different time intervals were added to 750 ml HNO_3 (purchased from Sigma-Aldrich). The solution was incubated for 5 days. After the incubation period 250 µl of digested sample was collected in a glass vial, and then 4750 µl of MiliQ was added to it forming a 5ml solution. ICP-MS (Agilent 7800 ICP-MS) was then carried out on these collected samples to quantify the

concentration of elements and determine the release levels of silver and titanium over time.

Confocal microscopy of catheter

Confocal microscopy was performed to check bacterial attached over the Ag-TiO_2 coated and uncoated catheter. Initially, the Ag-TiO_2 coated and uncoated catheter was incubated for 2 days with GFP-*E. coli* in LB media allowing biofilm to grow. After 2 days, the catheters were removed and the bacterial suspension were collected in eppendorf tubes. The bacterial suspensions were centrifuged for 10 minutes at 13,000 r.p.m. Following centrifugation, the supernatant were discarded and pellets were washed twice using PBS. 200 µl of *DAPI* (15 µg/ml) solutions was added to the pellet to visualize the attachment of both live and dead bacteria. The pellets were further washed utilizing PBS to eliminate unbound stain. 200 µl *PI* (10 µg/ml) was then added to the pellets to stain the dead bacterial cells. The pellets were again washed with the help of PBS to get rid of unbound dye. Thereafter, the pellets were collected and placed on a clean slide. Next, 2.5% glutaraldehyde (obtained from Sisco Research Laboratories Pvt. Ltd.) was applied to fix the pellets. It was left to dry for half an hour in the dark. Thereafter, images were captured employing Zeiss 510 Meta confocal microscopy system. ImageJ software was applied to count the total number of cells attached to the substrate.

Results

Thickness of Ag-TiO_2 thin film:

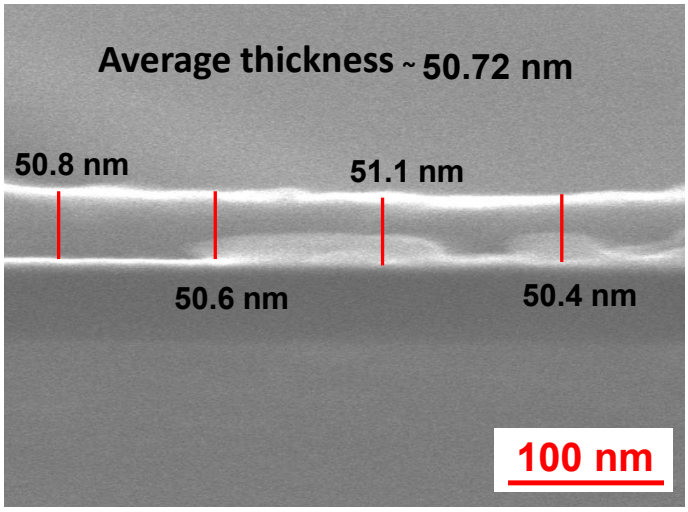

Figure S1. Cross-sectional FE-SEM image to determine the average Ag-TiO_2 film thickness.

EDX color mapping of various substrates:

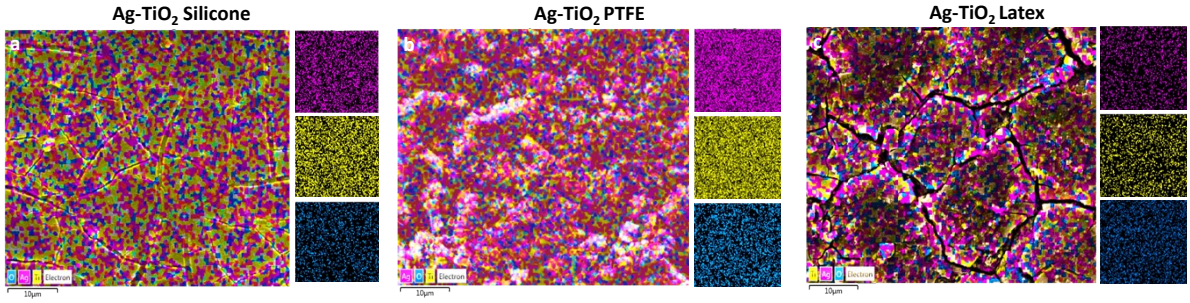

Figure S2. EDX color mapping image of Ag-TiO_2 coated **(a)** silicone, **(b)** PTFE, **(c)** latex.

Biocompatibility study

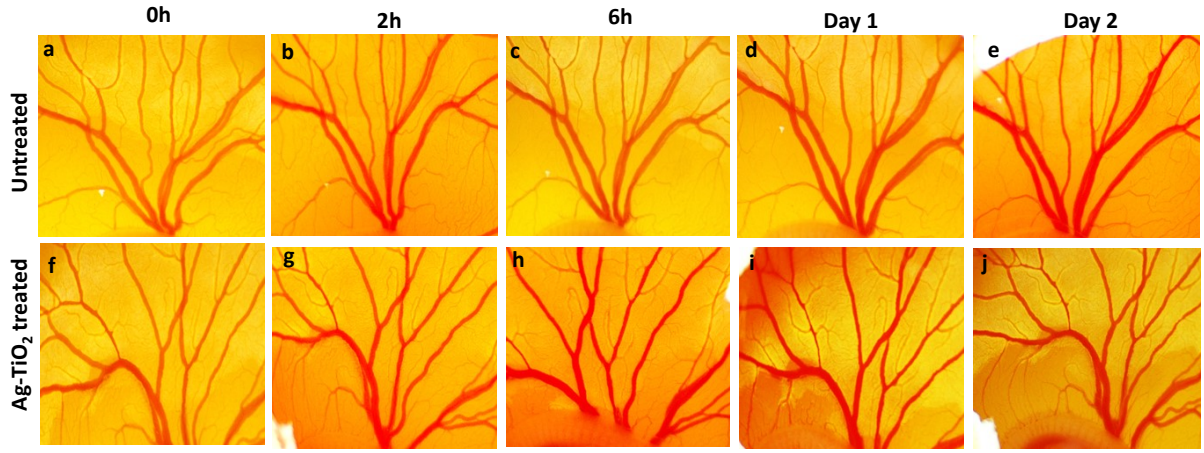

Figure S3. Microscopic images of (**a,b,c,d,e**) untreated and (**f,g,h,i,j,k**) Ag-TiO_2 treated chicken embryos were captured at various time points.

Confocal microscopy of catheters

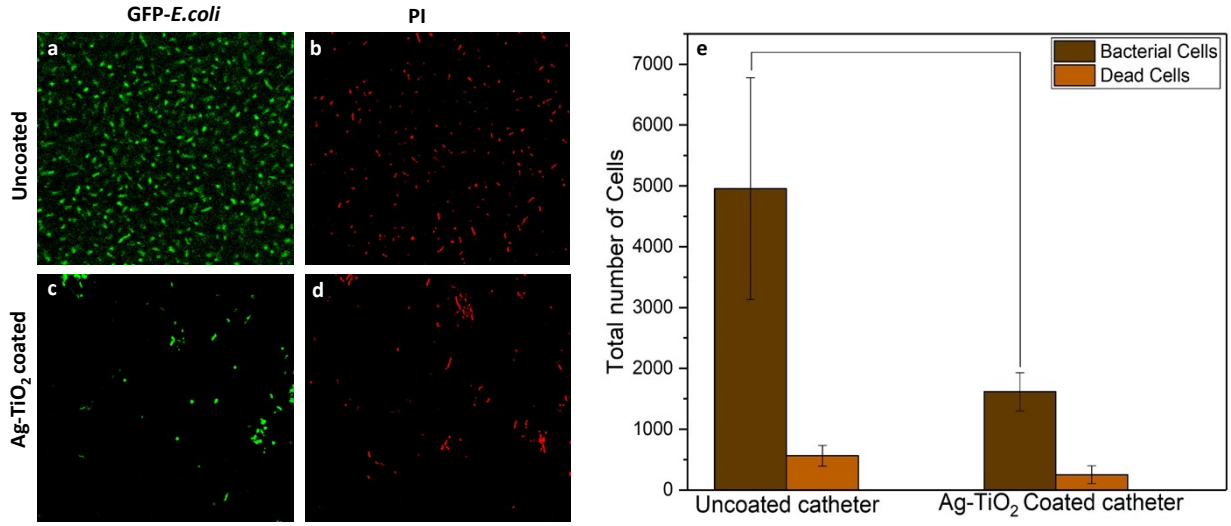

Figure S4. Confocal microscopy images of biofilm on (**a,b**) uncoated and (**c,d**) Ag-TiO_2 coated silicone catheters, **(e)** graph shows the total number of cells attached to the catheters. A significant difference was observed between untreated and Ag-TiO_2-treated samples. (*$p < 0.05$)

Stability study of Ag-TiO_2 coating over silicone and latex:

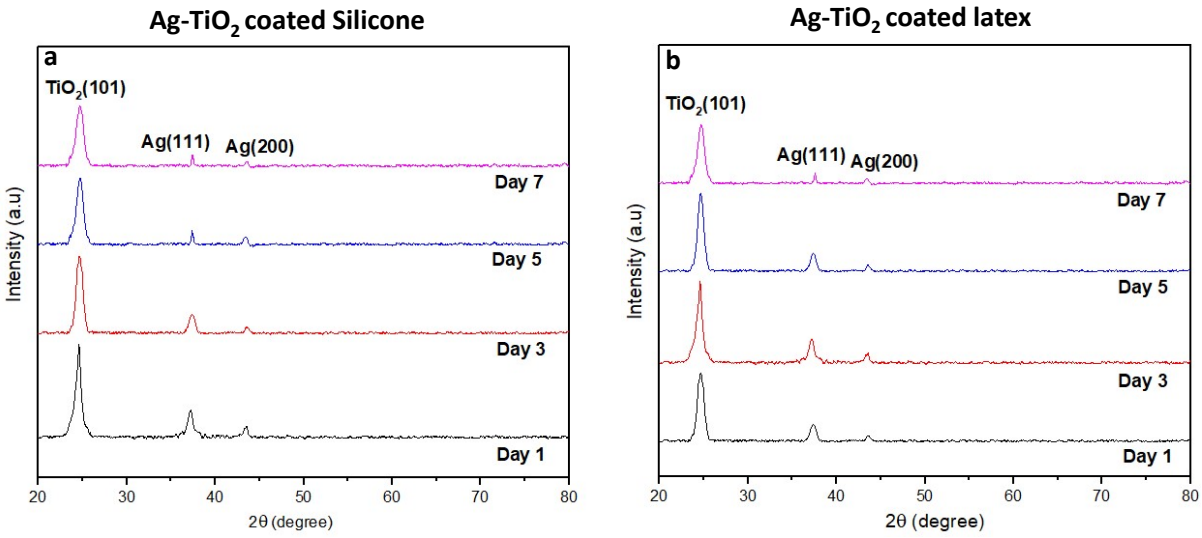

Figure S5. Stability study of consistent XRD peaks on **(a)** silicone and **(b)** latex substrate from day 1 to day 7.

Long-term stability study of Ag-TiO_2 coating over the glass substrate:

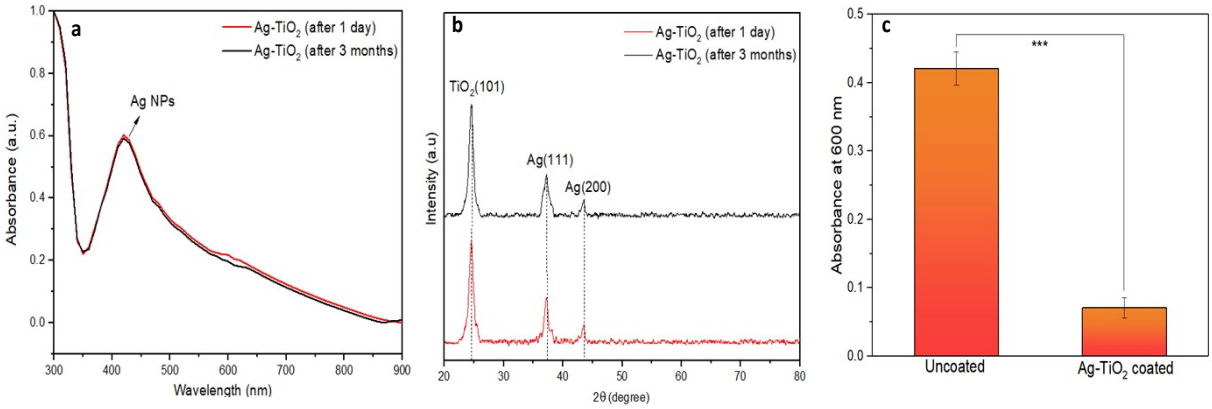

Figure. S6 Long-term stability data of (**a**) UV-Vis absorption and (**b**) XRD spectra of Ag-TiO_2 thin film on the glass substrate. (**c**) The absorbance of bacterial suspension exposed to uncoated and Ag-TiO_2 coated glass after incubation of 24h