Interaction of Red Blood Cells with Nanostructured surfaces

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Background:

- Titanium-based implants have good biocompatibility and are commonly used in blood contacting medical devices such as:

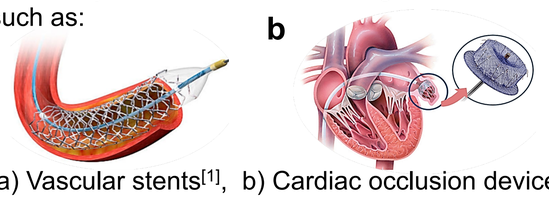


Figure 1: a) Vascular stents[1], b) Cardiac occlusion devices[2]

- When these implants come in contact with blood, platelet adhesion and activation occur, which may lead to further blood clotting and sometimes failure of these implants

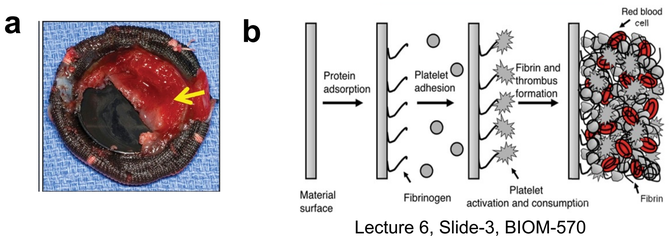


Figure 2: a) Thrombosis-affected artificial heart valve[3], b) Blood surface interaction

Why nanostructures:

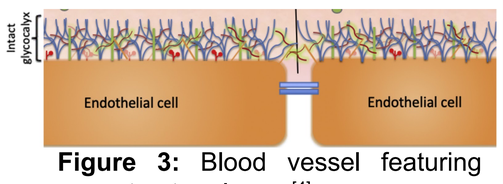


Figure 3: Blood vessel featuring nanostructure layers[4]

The blood contains cells on the micro-level. The vascular wall possess numerous nanostructured features (due to the presence of collagen and elastin in the vascular endothelial cellular matrix). Maintaining the nanoscale topography is desired because it mimics the natural tissue hierarchy, thus enhancing biocompatibility. The nanostructures increase the surface area and the surface area to volume ratio dramatically. This allows the cells to interact with the surface.

Fabrication and Modification of nanostructured surfaces:

Nanotubes (NT)

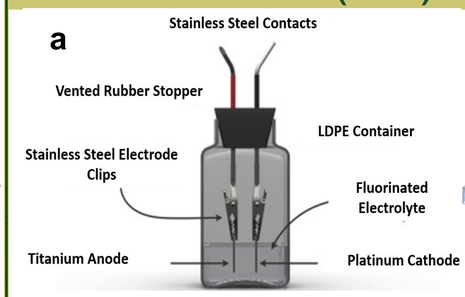
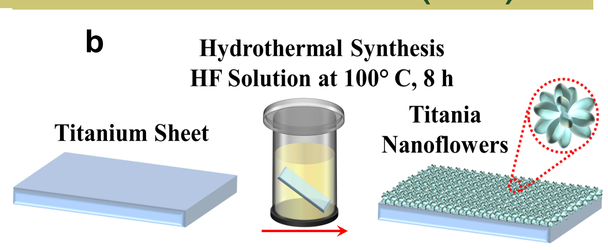


Figure 4: a) Nanotubes were prepared using an anodization process. In this process, titanium was used as an anode and platinum was used as a cathode. 55 V were applied for 22 hours to form the NT.

Nanoflowers (NF)



b) The NF were prepared by adding 0.08% of hydrofluoric acid in deionized water. The titanium substrate was added to the Polytetrafluoroethylene (PTFE) container and heated using a hot plate for 8 hours at 300℃.

The nanostructured surfaces were modified using heptadecafluoro-(1,1,2,2-tetrahydro decyl) trichlorosilane (FL). This was performed to make the surfaces superhydrophobic. The substrates were positioned on a hot plate near a glass slide with 150 µl of FL. The glass slide and substrates were covered with a bowl and heated at 120 °C for 1 h.

Isolation of RBCs: Human blood was collected in EDTA coated blood collection vials. Serum proteins, leukocytes, and platelets were removed via aspiration and red blood cells (RBCs) were resuspended with Dulbecco's phosphate buffered saline solution (PBS; pH 7.4) to a final concentration of 10^7 cell/ml.

Results:

Characterization:

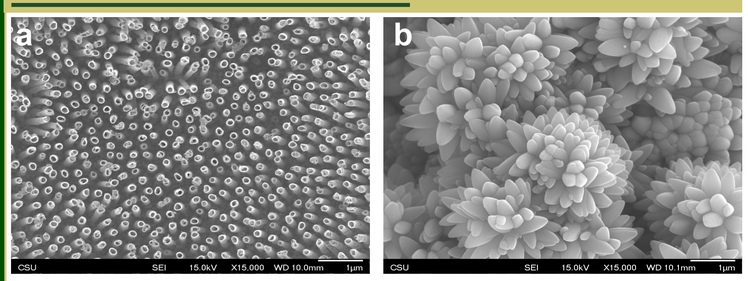
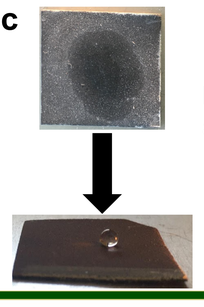
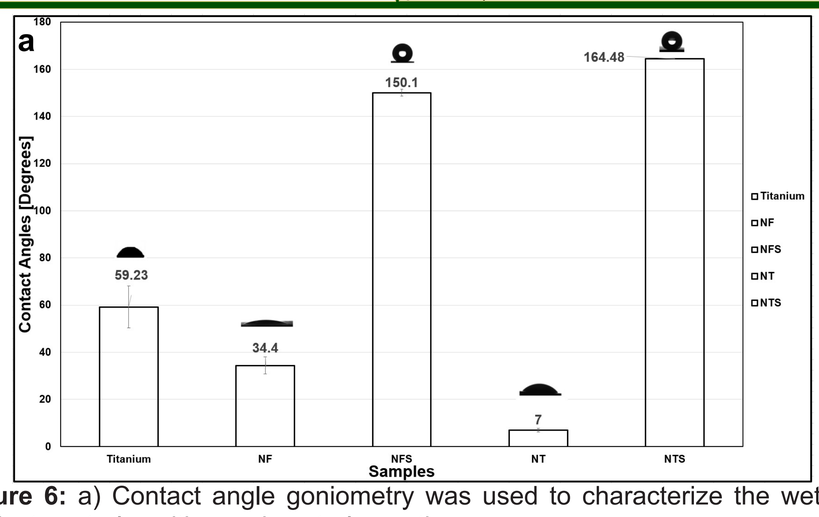


Figure 5: a) Nanotubes, b) Nanoflowers, c) Difference between NF and Nanoflowers treated with silane (NFS)

The modified surfaces were compared to the unmodified surfaces using scanning electron microscopy (SEM). There was no difference with the surface morphology, but the surface chemistry changed from hydrophilic to superhydrophobic.





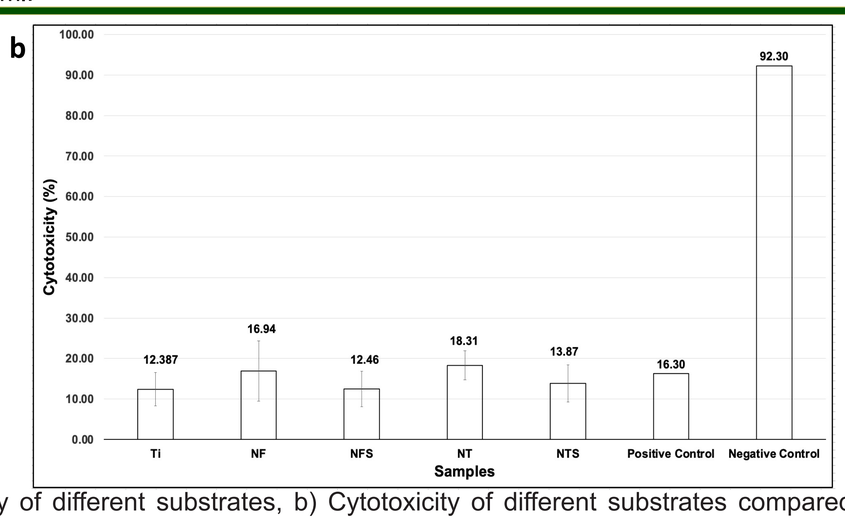


Figure 6: a) Contact angle goniometry was used to characterize the wettability of different substrates, b) Cytotoxicity of different substrates compared against control positive and control negative

Static contact angles were measured using DI water. When the static contact angle (θ) between the surface and DI water droplet is greater than 150°, the surface is designated superhydrophobic. The contact angles for NFS and nanotubes treated with silane (NTS) were greater than 150 degrees. Cell cytotoxicity for RBCs exposed to different surfaces was measured using the lactate dehydrogenase (LDH) assay. The results indicate no significant differences in the LDH activity on all the substrates and positive control (100% live cells), whereas the LDH activity for the negative control (100% dead cells) was significantly different than all the other substrates.

Nanostructured Surfaces (Fluorescence microscopy)

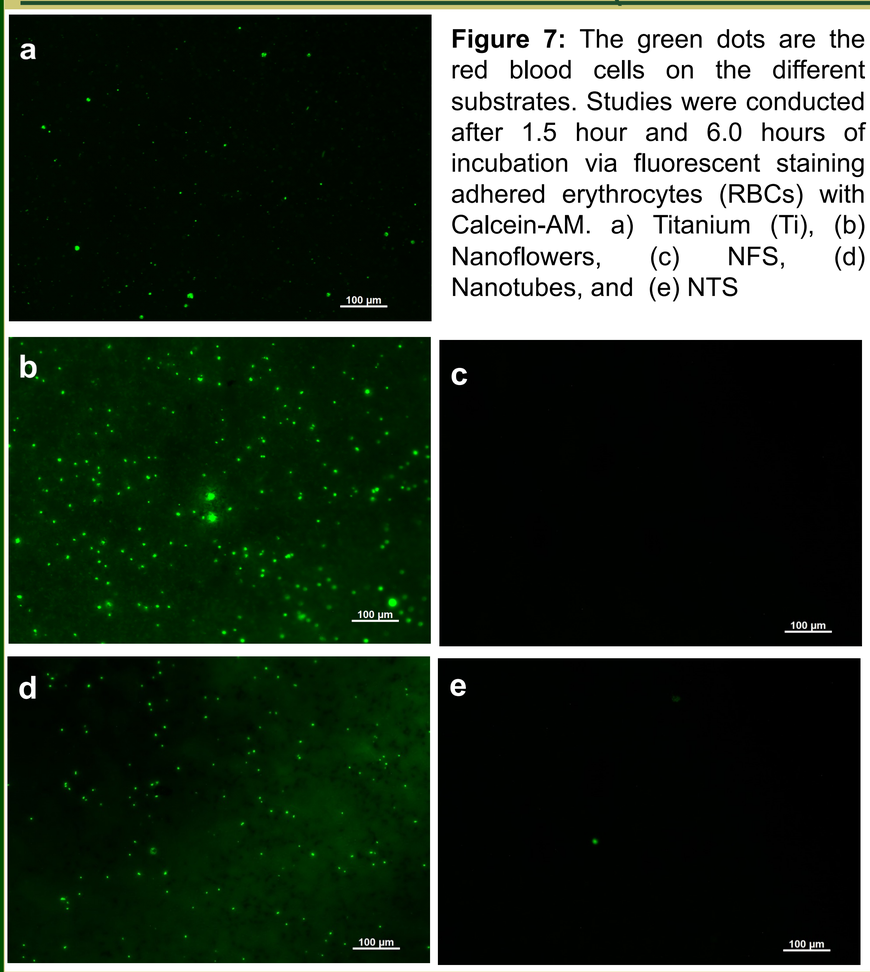


Figure 7: The green dots are the red blood cells on the different substrates. Studies were conducted after 1.5 hour and 6.0 hours of incubation via fluorescent staining adhered erythrocytes (RBCs) with Calcein-AM. a) Titanium (Ti), (b) Nanoflowers, (c) NFS, (d) Nanotubes, and (e) NTS

Table 1: Surface area of substrates covered by RBCs

Substrates	Mean Area
Ti	2.137
NF	0.641
NFS	0.429
NT	1.100
NTS	0.443

The RBCs didn't adhere to the substrates treated with FL (c and e) compared to the untreated surfaces. RBCs on the titanium surface (a) were aggregated (stacked up) which is one of the reasons for the blood clot. There is significant reduction in RBC adhesion on NFS (c) and NTS (e) compared to titanium, which is supported by the data in Table 1.

Nanostructured Surfaces (Scanning electron microscopy)

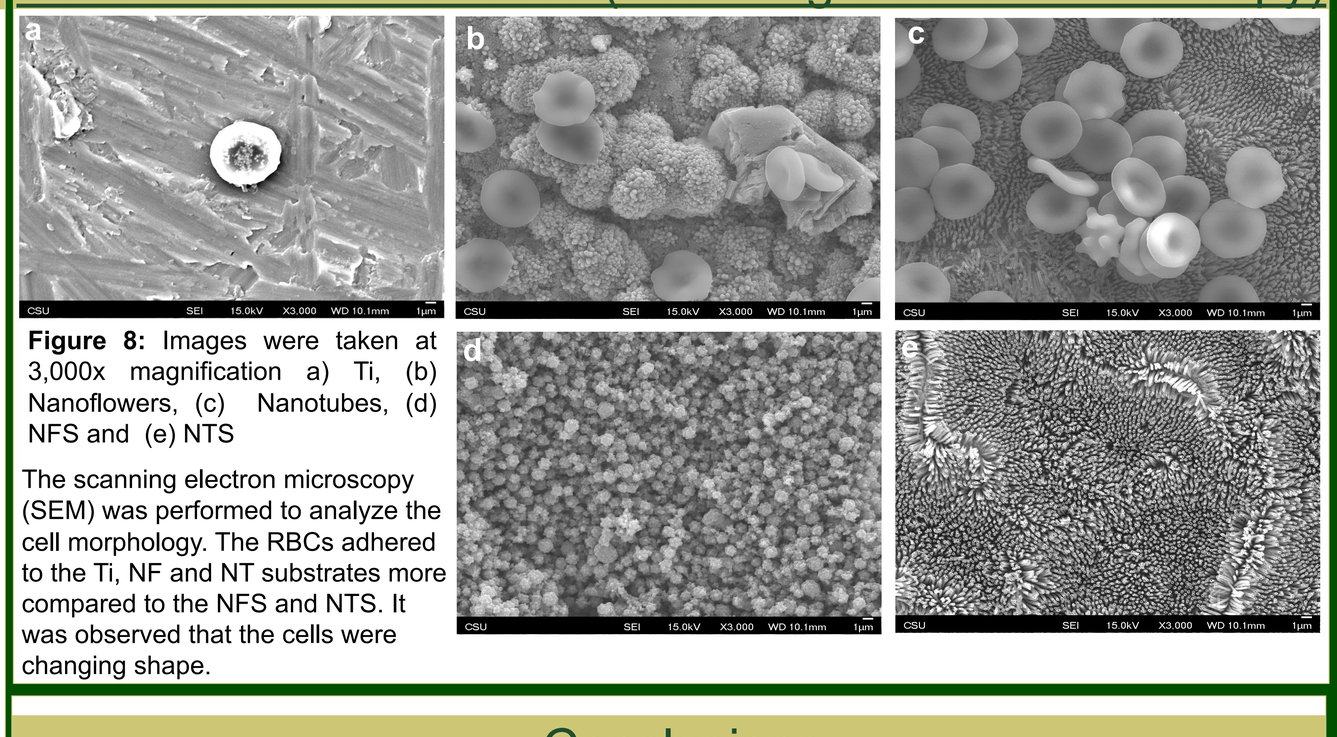


Figure 8: Images were taken at 3,000x magnification a) Ti, (b) Nanoflowers, (c) Nanotubes, (d) NFS and (e) NTS

The scanning electron microscopy (SEM) was performed to analyze the cell morphology. The RBCs adhered to the Ti, NF and NT substrates more compared to the NFS and NTS. It was observed that the cells were changing shape.

Conclusions

- The fabricated and modified surfaces were found to be superhyrophobic.
- When compared to NF and NT surfaces, the NFS and NTS exhibits the inhibition of the adherence of RBCs. The results shown are only for 1.5 hours of incubation.
- There were morphological changes in the cells when incubated for 1.5 and 6 hours.
- The fabricated and modified surfaces are not toxic to the RBCs.

Future Directions

- Analyze the change in shape of the RBCs cells due to the nanostructured surfaces.
- Analyze the interaction of RBCs with the nanostructured surfaces for 24 hours.
- Later, the interaction of whole blood with the nanostructured surfaces will be analyzed.

Acknowledgements:

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References:

[1] "vascular stents - Google Search." https://www.google.com/search?q=vascular+stents&sxsrf=ALeKk01SqzhuV_5ICfUazqYR87Hnalat-Q:1618697005900&source=lnms&tbm=isch&sa=X&ved=2ahUKEwiygKSgpIbwAhUJOs0KHYjiBHcQ_AUoAXoECAEQAw&biw=1200&bih=722&dpr=2#imgrc=Bu-dMjZqHLSwAM (accessed Apr. 17, 2021).

[2] "Left Atrial Appendage Closure Procedures | Johns Hopkins Medicine." https://www.hopkinsmedicine.org/health/treatment-tests-and-therapies/left-atrial-appendage-closure-procedures (accessed Apr. 17, 2021).

[3] "Prosthetic valve thrombosis: Time is critical - Mayo Clinic." https://www.mayoclinic.org/medical-professionals/cardiovascular-diseases/news/prosthetic-valve-thrombosis-time-is-critical/mac-20430866 (accessed Apr. 17, 2021).

[4] "The Pathological Relevance of Increased Endothelial Glycocalyx Permeability - The American Journal of Pathology." https://ajp.amjpathol.org/article/S0002-9440(20)30073-0/fulltext (accessed Apr. 18, 2021).