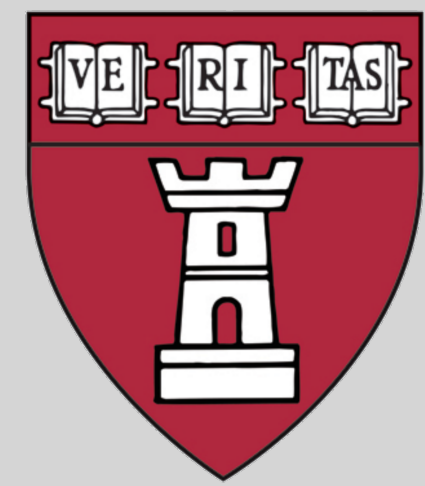




Plasma Treatment Removes Implant Surface Contaminants and Improves Biocompatibility

Kailing Ho¹, Chia-Yu Chen¹, Takahiko Shiba¹, David M. Kim¹

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Introduction:

Surface modification of titanium implant and abutment has been extensively studied to improve the longevity and long-term outcome of implant therapy. Plasma treatment on titanium surfaces has the potential of becoming a chair-side treatment to bio-activate and regenerate titanium surfaces prior to implant surgery. This *in-vitro* study aims to evaluate the effects of plasma treatment on titanium surfaces and the cellular response of murine fibroblasts and osteoblasts on treated surfaces.

Materials and Methods:

Machined and rough-surfaced titanium discs were treated with plasma and subsequently evaluated for architectural changes under scanning electron microscopy (SEM) and elemental changes under energy dispersive spectroscopy (EDS). The discs were also evaluated for changes in surface hydrophilicity using contact angle analysis. To assess the cellular response of fibroblast and osteoblasts on treated surfaces, the corresponding cells were seeded for 1, 2, 6, 12, and 24 hours to observe adhesion and proliferation quantitatively. Subsequent qualitative analysis was done immunohistochemistry staining and SEM imaging to observe cell morphology in response to treatment. Statistical analyses were conducted using one-tailed unpaired t-test at 95% CI.

Results:

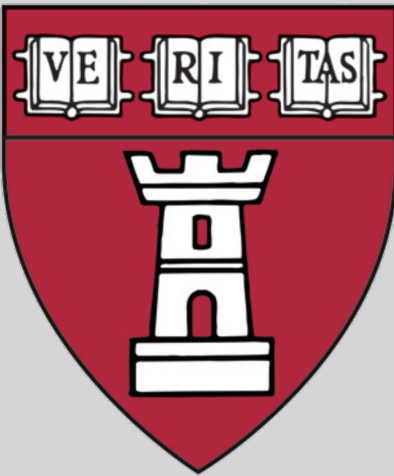
Plasma treatment showed minimal to no surface changes based on SEM imaging. There was, however, a notable decrease in the amount of carbon buildup on the discs after plasma treatment. Plasma also drastically increased hydrophilicity of the titanium surface. Cell adhesion to the titanium surfaces also improves with plasma treatment, with fibroblast cells reaching statistical significance ($p < 0.05$) in favor of plasma treatment at 1, 2, and 6-hour timepoints. Similar findings were seen with osteoblasts, with statistical significance noted at 1 and 2-hour timepoints. Under immunohistochemistry and SEM imaging, osteoblast and fibroblast morphology shows greater spread of cytoskeleton, which suggests an “activated” state of both cell types.

Conclusion:

Plasma treatment does not significantly alter implant surface architecture. It does, however, significantly improve surface hydrophilicity and improves cell adhesion during early time points. Plasma treatment also seems to provide a surface that is beneficial for cell activation and boost early stages of implant healing compared to control samples. The results of the study demonstrated that plasma treatment has the potential to be an effective chair-side treatment to improve surface biocompatibility, early implant healing, and possibly long-term implant outcomes.

**Plasma
treatment on
titanium disc**





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RESULTS

Plasma treatment does not significantly alter titanium surface architecture while reducing carbon build-up on surfaces

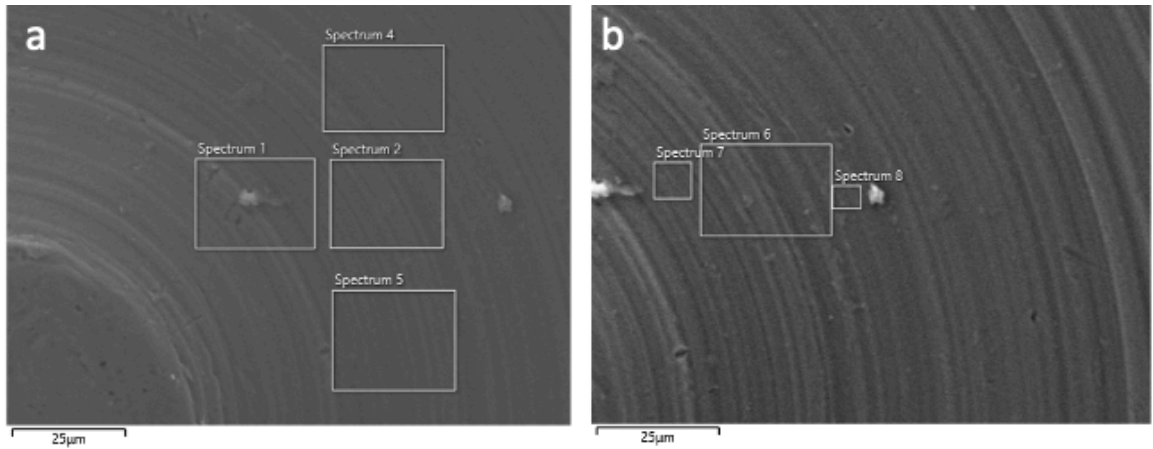


Figure 1a. Machined surface (control - no plasma) at 1000x magnification. Note the two points of contamination as reference point for SEM imaging.

Figure 1b. Machined surface (control - after plasma) at 1000x magnification.

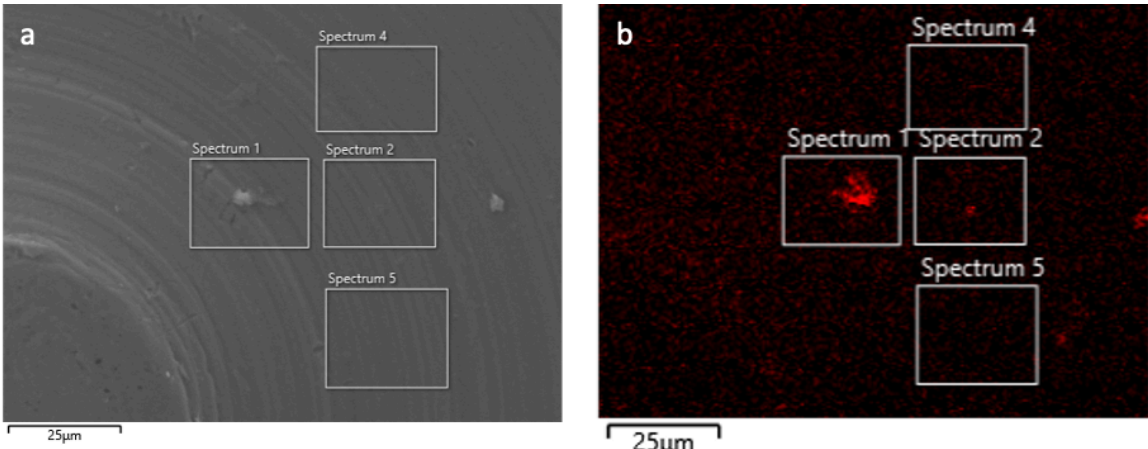
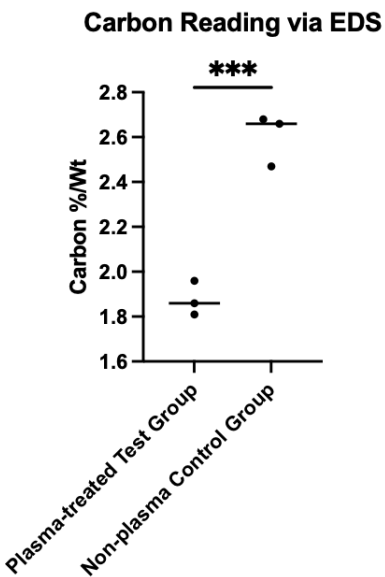


Figure 2a. Machined surface imaged with SEM. Note the presence of surface contaminants.

Figure 2b. Machined surface imaged with EDS for carbon species. The surface contaminants have a high build-up of carbon. There is also an abundance of carbon species across the rest of the disc



Plasma treatment increases titanium surface hydrophilicity

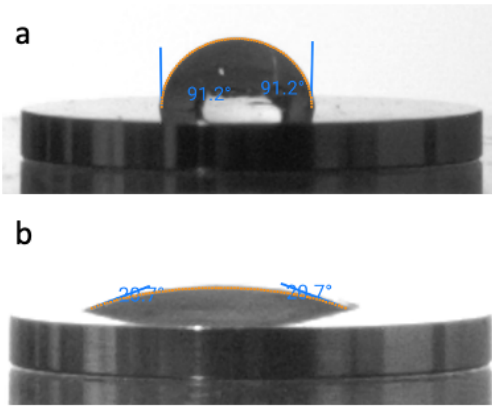
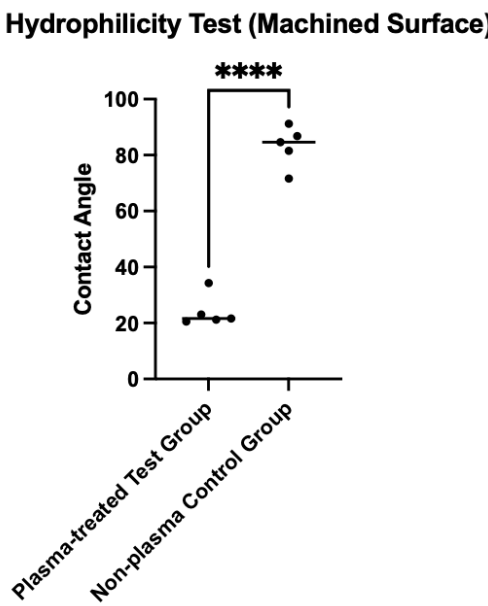


Figure 3a, 3b. Contact angle of Milli-Q water on machined titanium surface before (a) and after (b) plasma treatment. Before treatment, left and right contact angles are 91.2°. After plasma treatment, left and right contact angles are 20.7°.

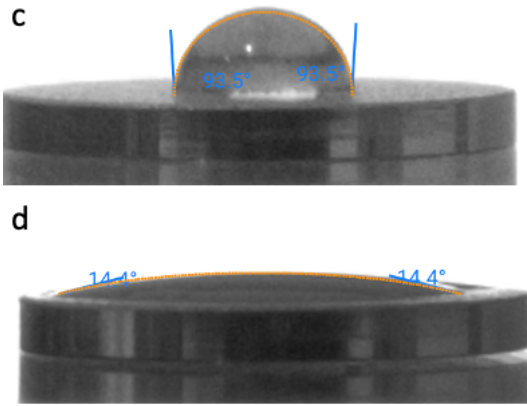
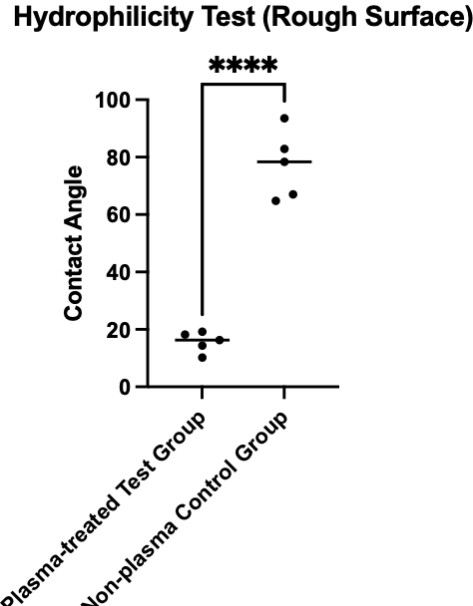


Figure 3c, 3d. Contact angle of Milli-Q water on rough-surfaced titanium disc before (c) and after (d) plasma treatment. Before treatment, left and right contact angles are 93.5°. After plasma treatment, left and right contact angles are 14.4°.

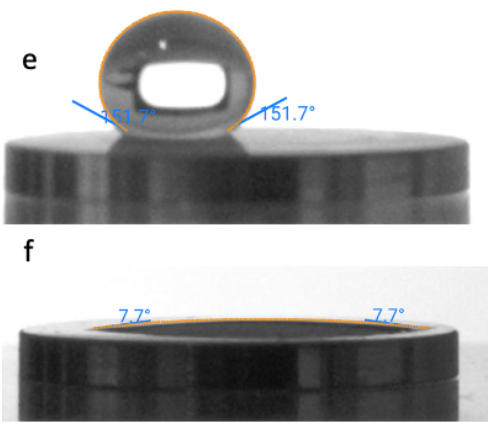
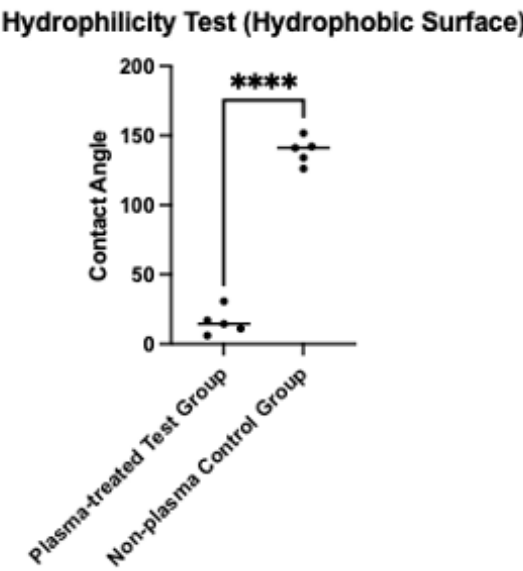
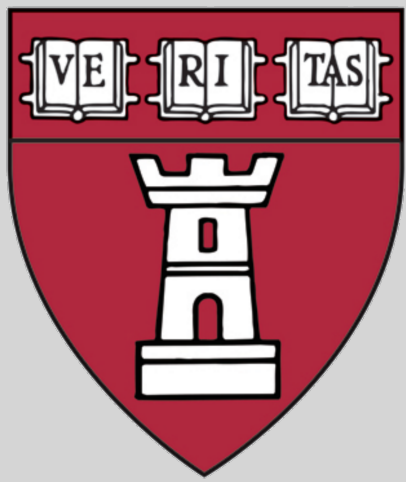


Figure 3e, 3f. Contact angle of Milli-Q water on hydrophobic titanium surface before (e) and after (f) plasma treatment. Before treatment, left and right contact angles are 151.7°. After plasma treatment, left and right contact angles are 7.7°.





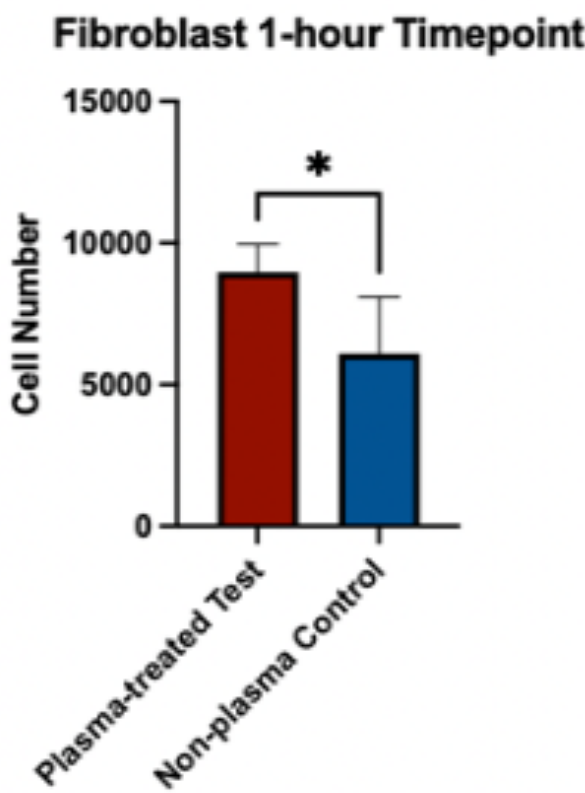
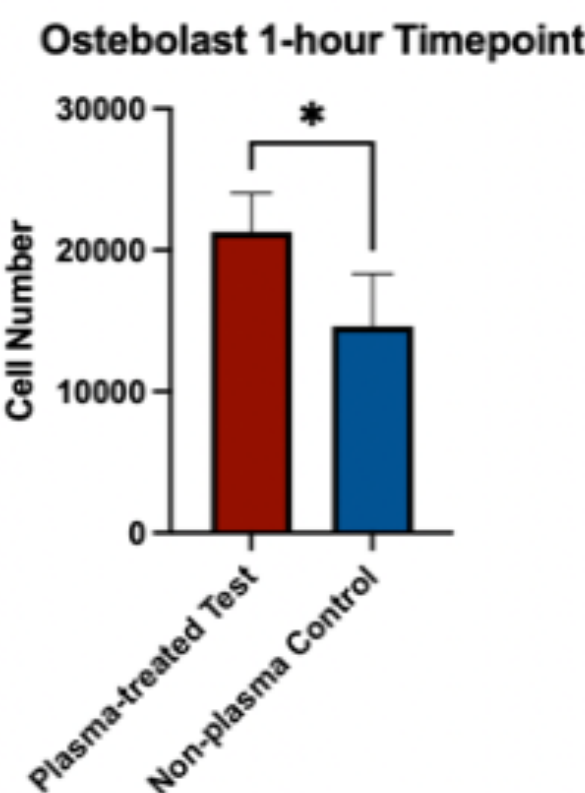
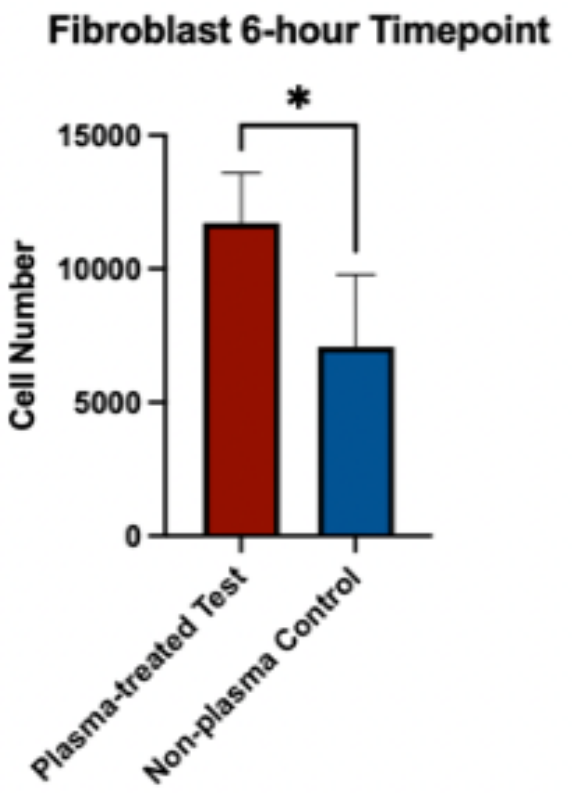
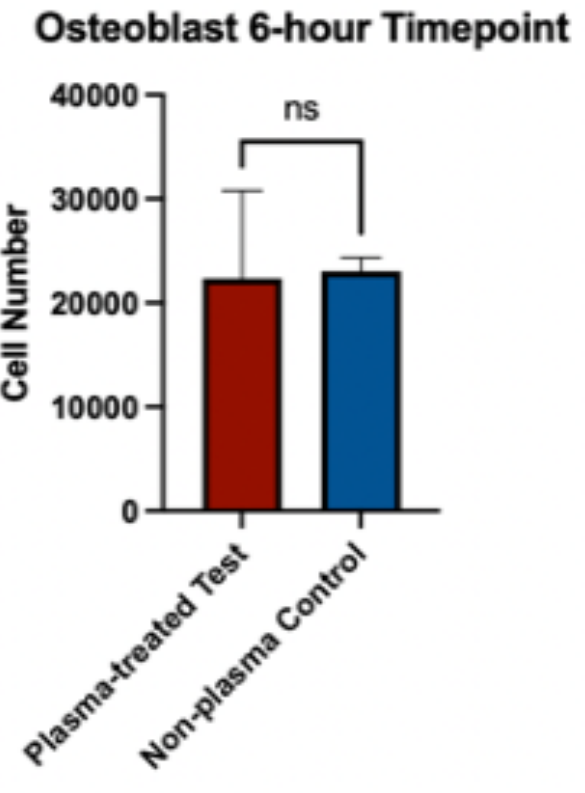
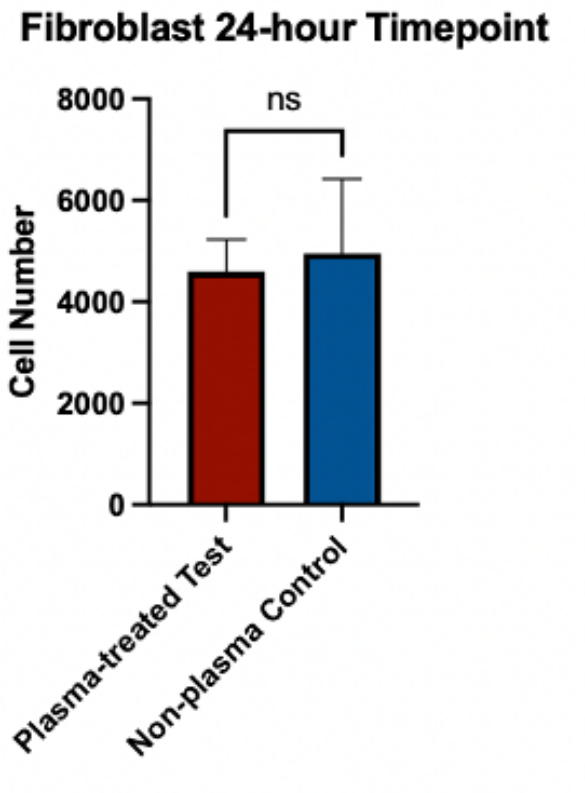
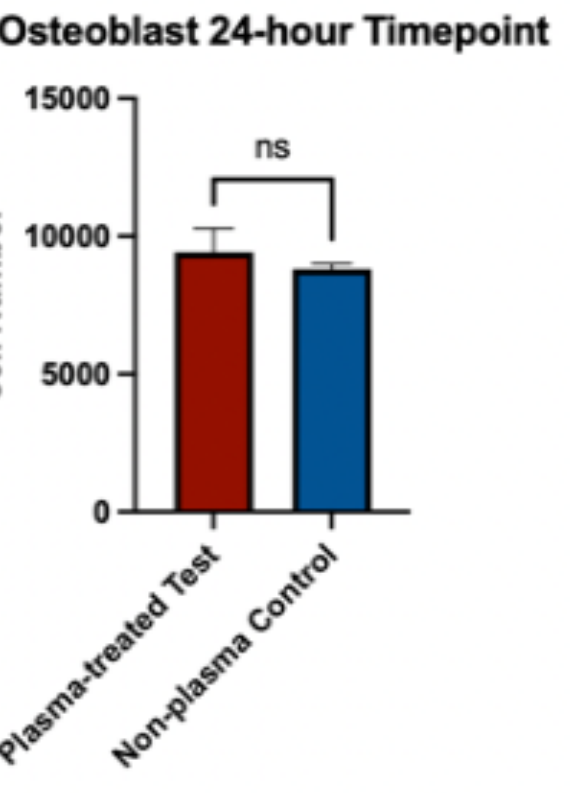
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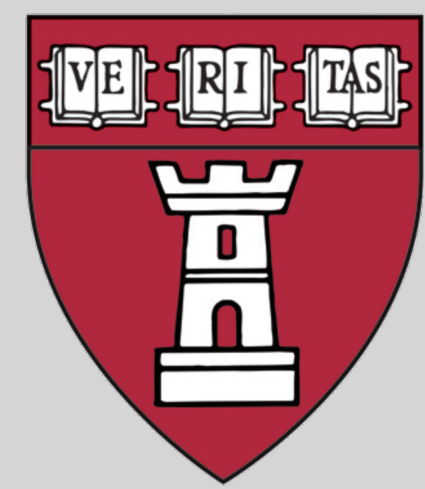
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RESULTS

Fibroblast and osteoblast cells cultured on plasma-treated titanium surfaces have better after cell seeding during early phases





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RESULTS

Osteoblast cells cultured on plasma-treated titanium surfaces display “activated” cell morphology

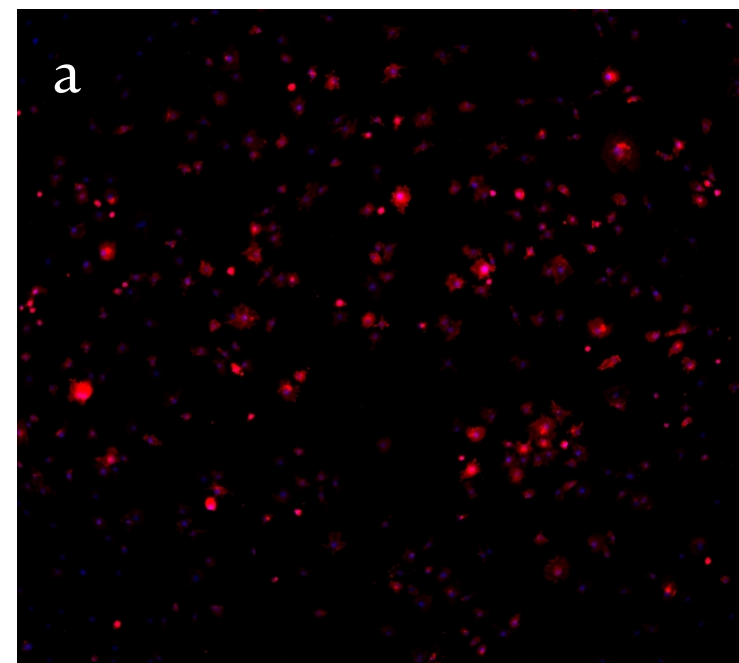
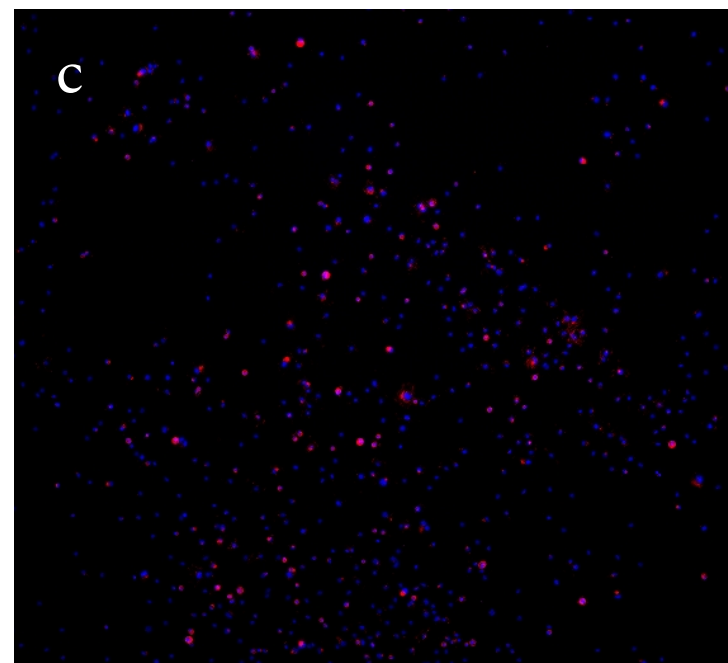
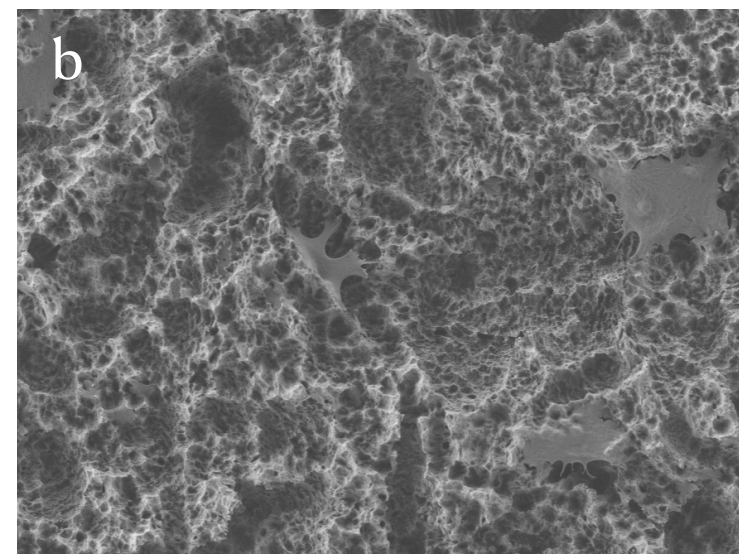
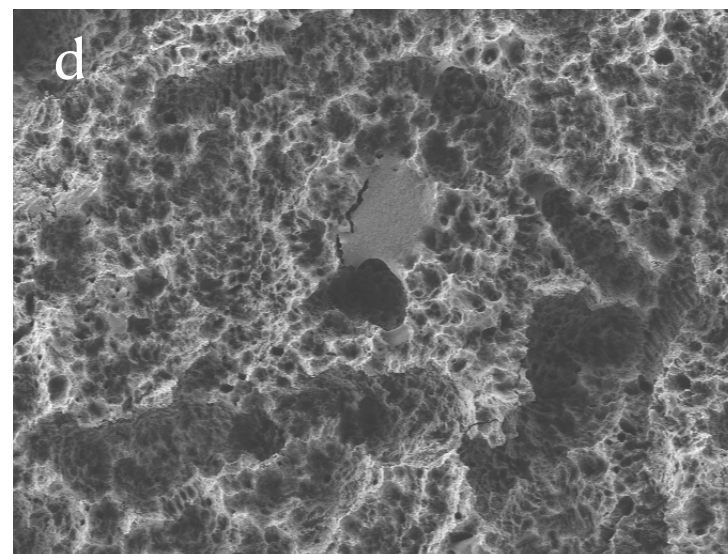


Figure 4a-b. Immunohistochemical staining (DAPI and Phalloidin) of osteoblast cells at 1-hour time point. Imaged at 4x magnification.

Figure 4c-d. SEM imaging of osteoblast cells on tough titanium surface at 1-hour time point. Magnification at 5000x. Plasma-treated test group on the right, and non-plasma control group on the left.

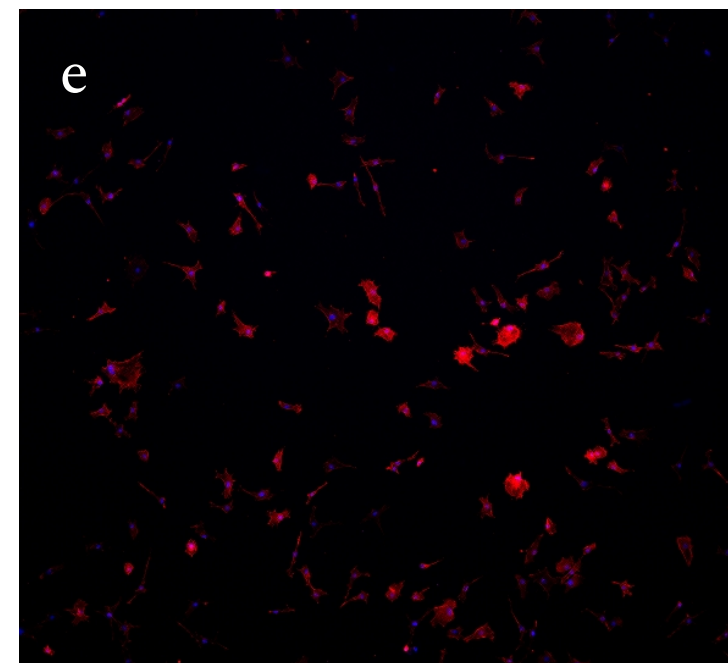
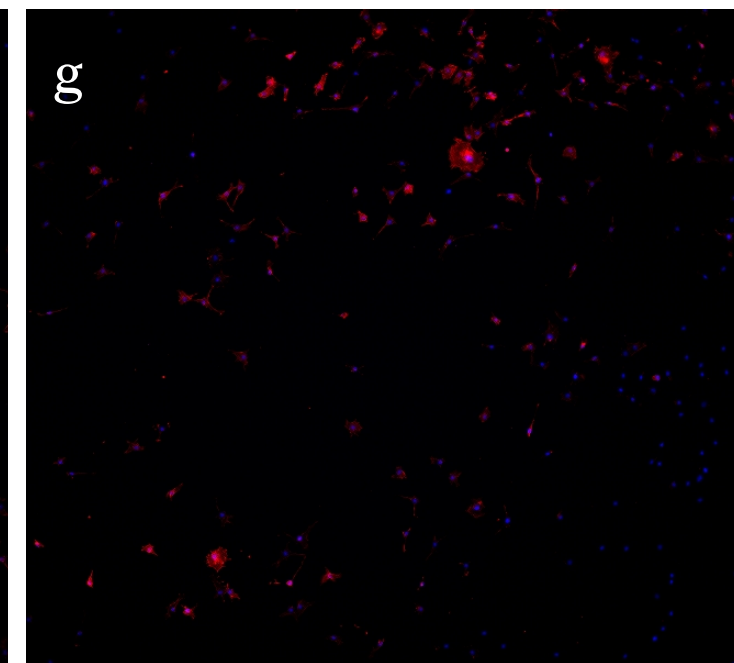
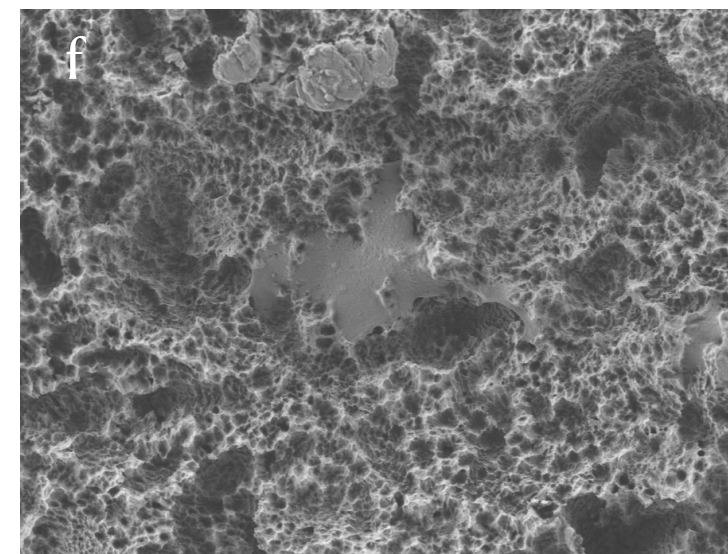
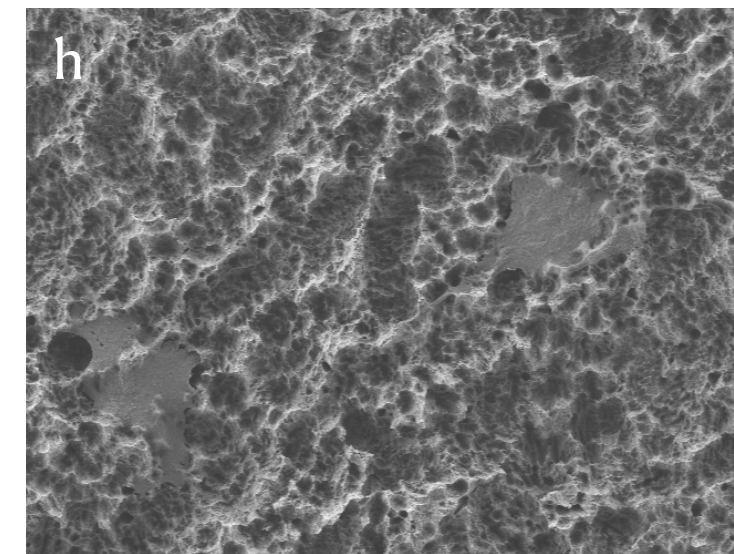


Figure 4e-f. Immunohistochemical staining (DAPI and Phalloidin) of osteoblast cells at 6-hour time point. Imaged at 4x magnification.

Figure 4g-h. SEM imaging of osteoblast cells on tough titanium surface at 6-hour time point. Magnification at 5000x. Plasma-treated test group on the right, and non-plasma control group on the left.

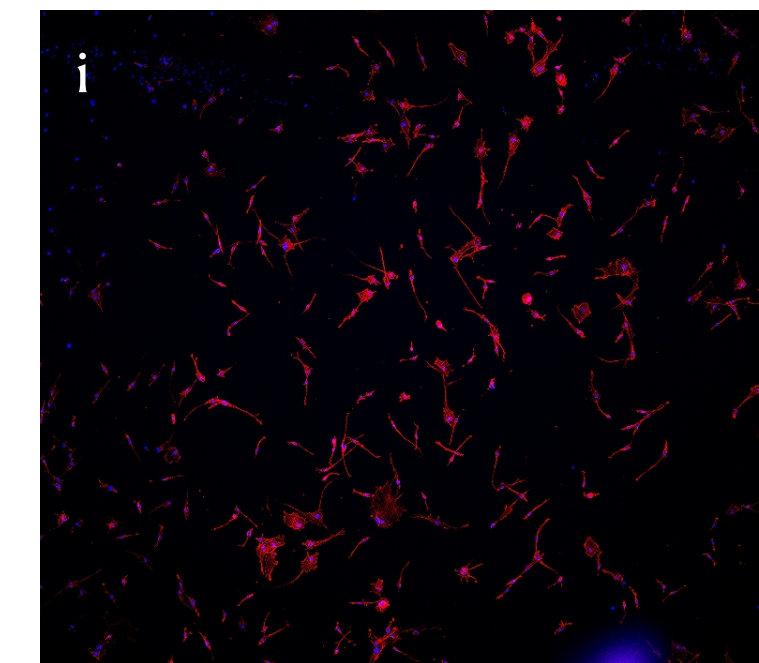
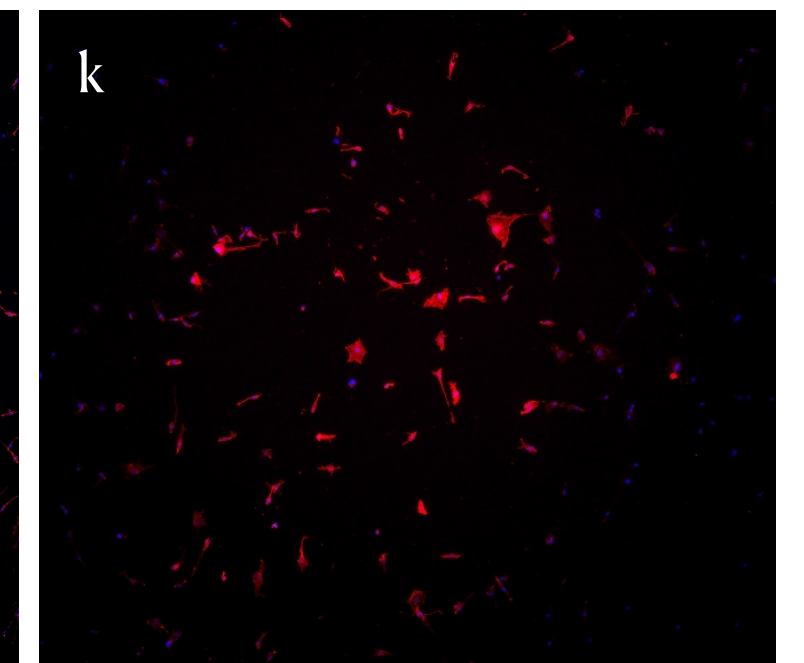
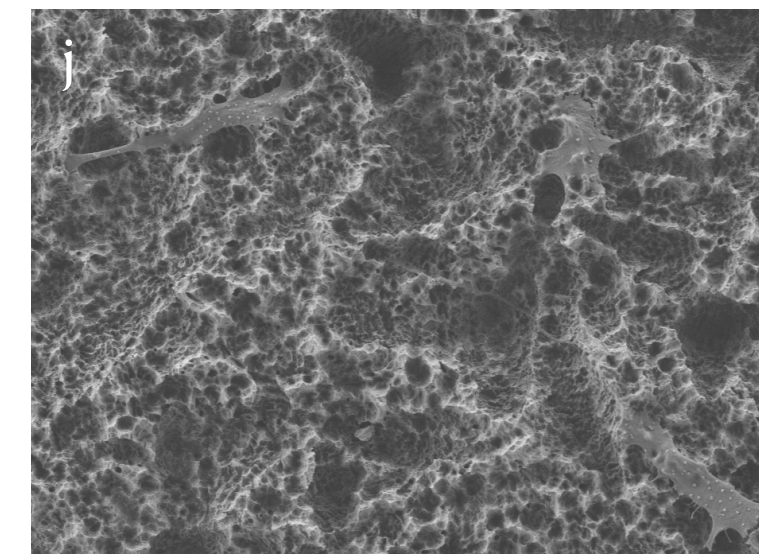
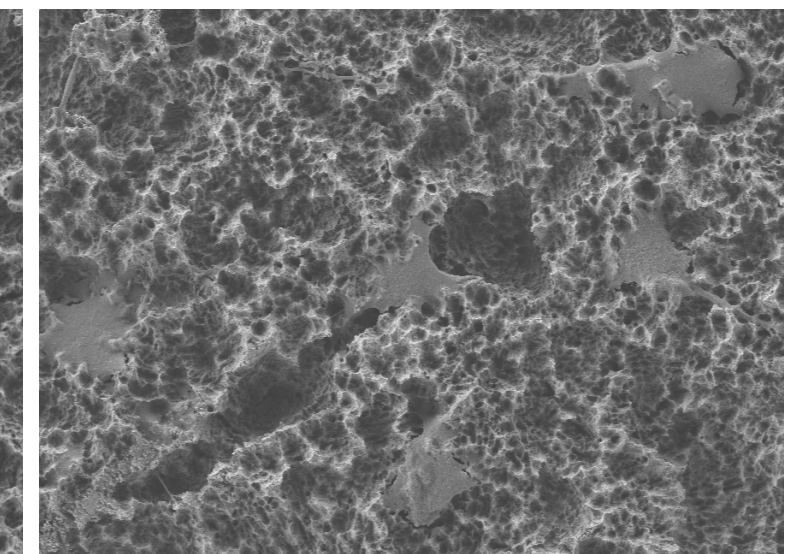
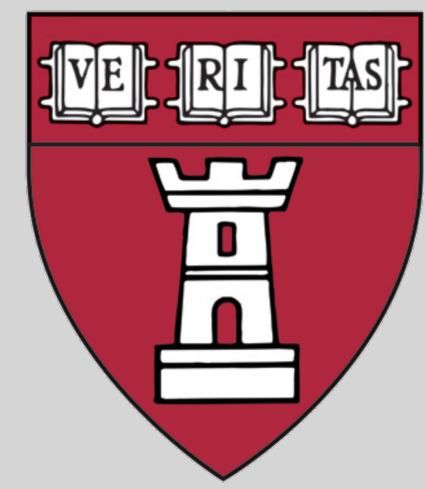


Figure 4i-j. Immunohistochemical staining (DAPI and Phalloidin) of osteoblast cells at 24-hour time point. Imaged at 4x magnification.

Figure 4k-l. SEM imaging of osteoblast cells on tough titanium surface at 24-hour time point. Magnification at 5000x. Plasma-treated test group on the right, and non-plasma control group on the left.



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RESULTS

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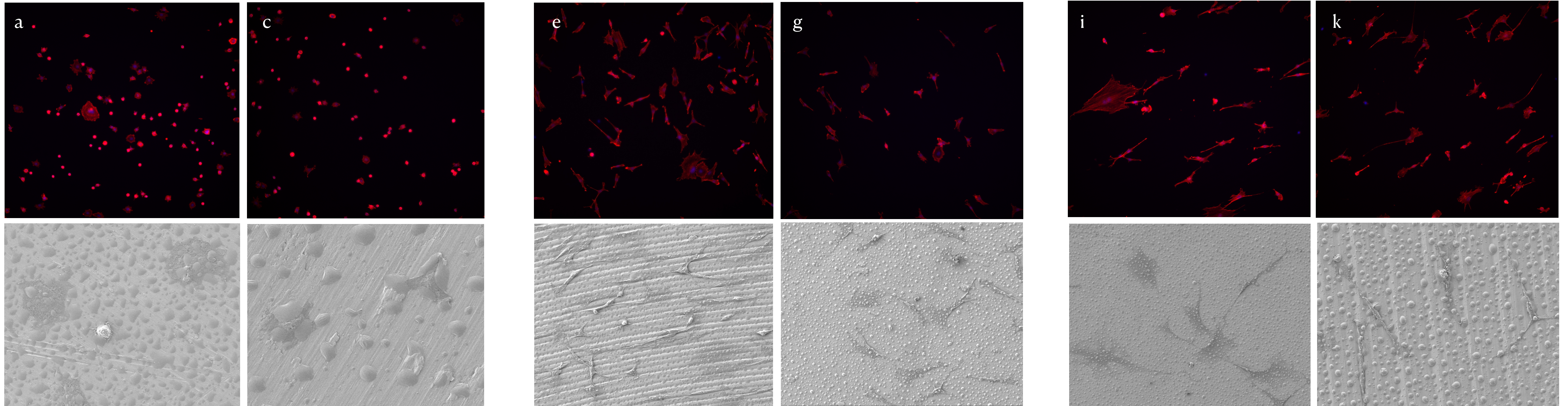


Figure 5a-b. Immunohistochemical staining (DAPI and Phalloidin) of fibroblast cells at 1-hour time point. Imaged at 10x magnification.

Figure 5c-d. SEM imaging of fibroblast cells on machined titanium surface at 1-hour time point. Magnification at 5000x. Plasma-treated test group on the right, and non-plasma control group on the left.

Figure 5e-f. Immunohistochemical staining (DAPI and Phalloidin) of fibroblast cells at 6-hour time point. Imaged at 10x magnification.

Figure 5g-h. SEM imaging of fibroblast cells on machined titanium surface at 6-hour time point. Magnification at 5000x. Plasma-treated test group on the right, and non-plasma control group on the left.

Figure 5i-j. Immunohistochemical staining (DAPI and Phalloidin) of fibroblast cells at 24-hour time point. Imaged at 10x magnification.

Figure 5k-l. SEM imaging of fibroblast cells on tough titanium surface at 24-hour time point. Magnification at 5000x. Plasma-treated test group on the right, and non-plasma control group on the left.