

Appendix A: SEM Images

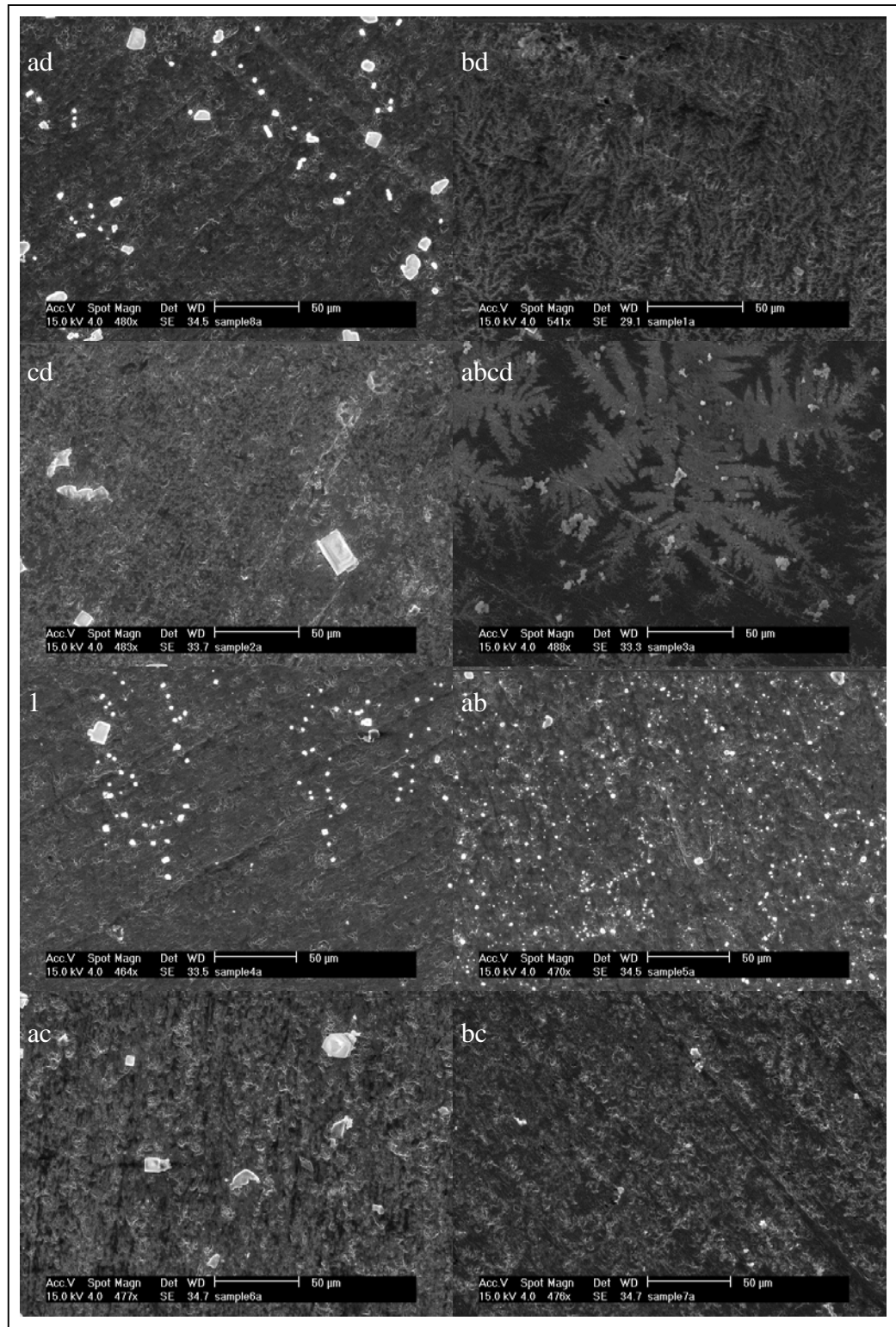


Figure A1: SEM images from the one-half fractional factorial experiments (the first replicate).

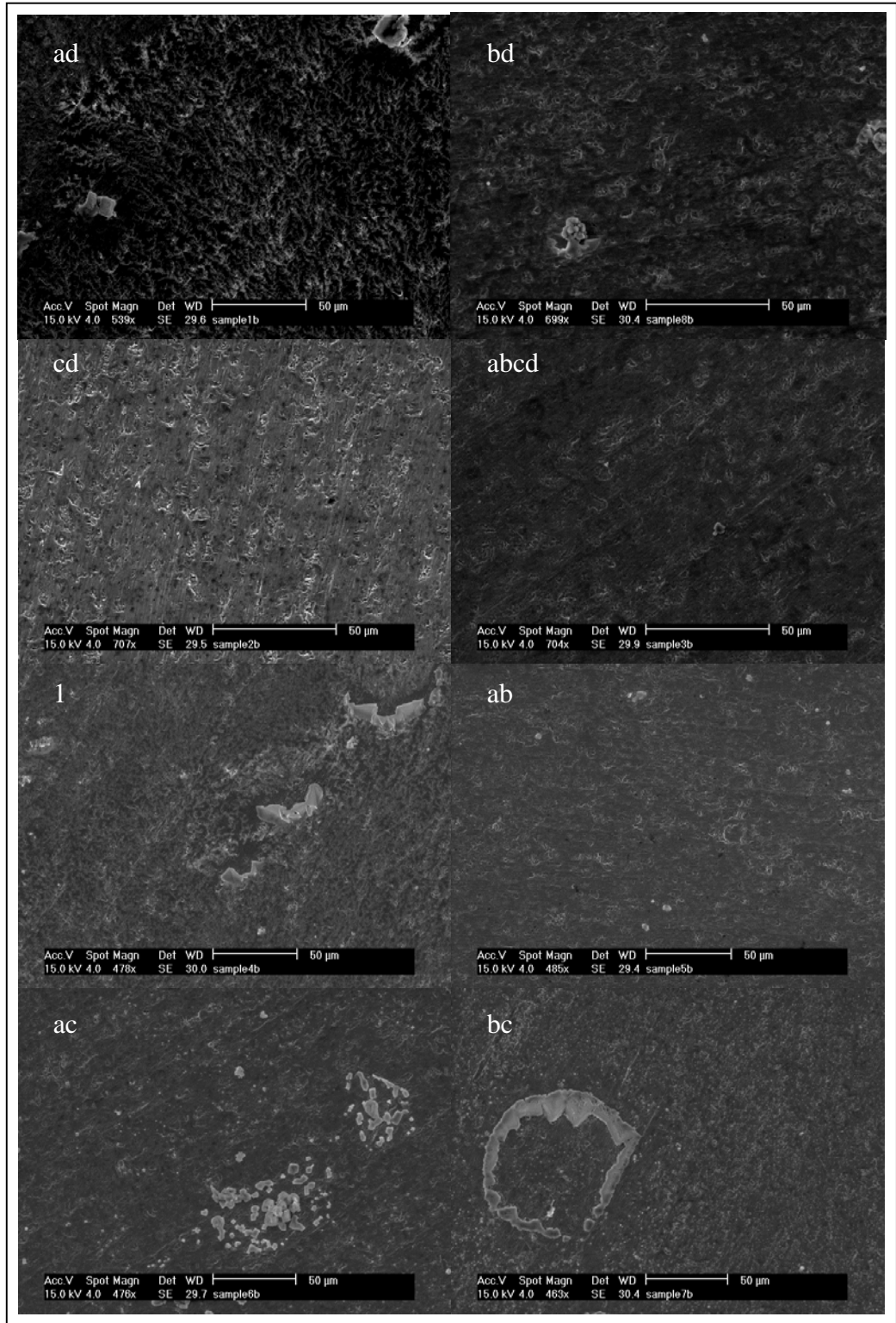


Figure A2: SEM images from the one-half fractional factorial experiments (the second replicate).

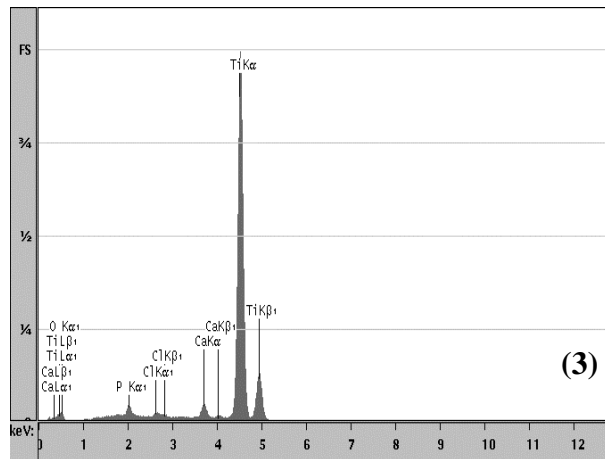
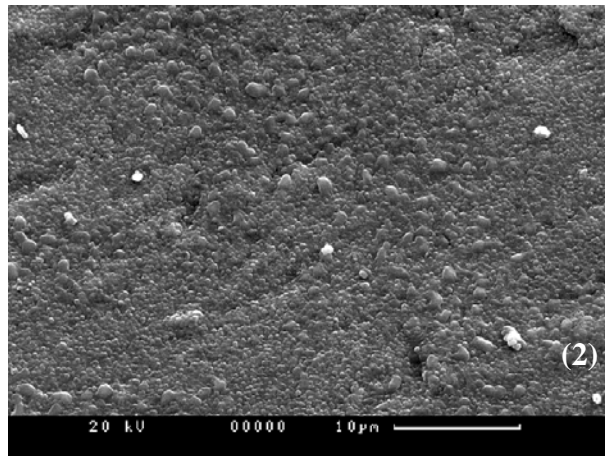
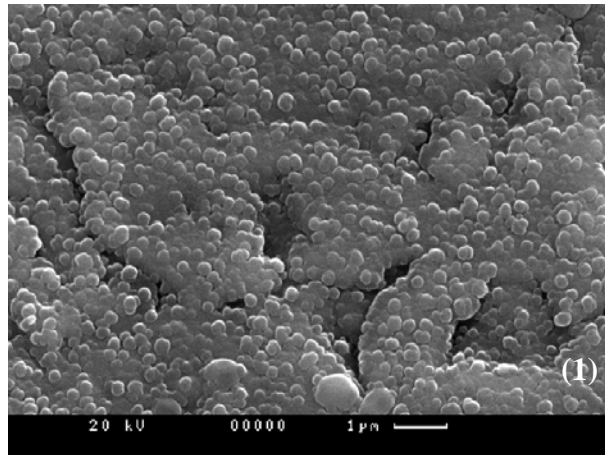


Figure A3: SEM images at 1 μm (1) and 10 μm (2) and EDS spectra (3) of HA coating on titanium following immersion in SBF for 5 days.

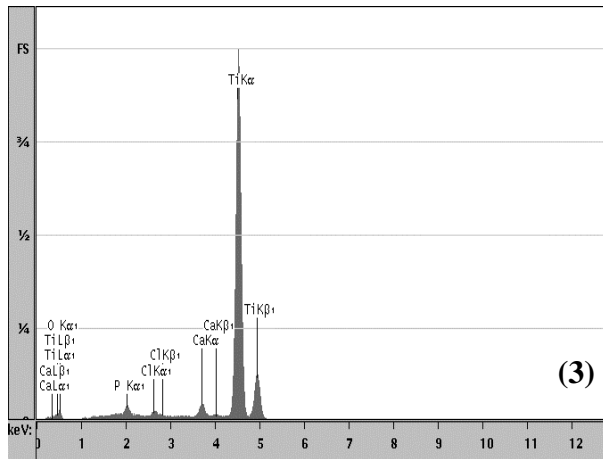
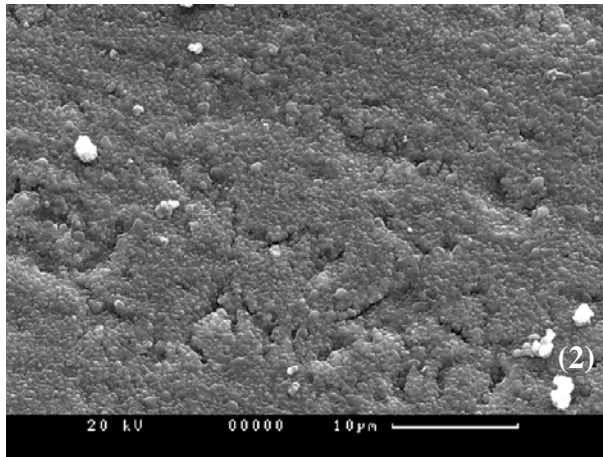
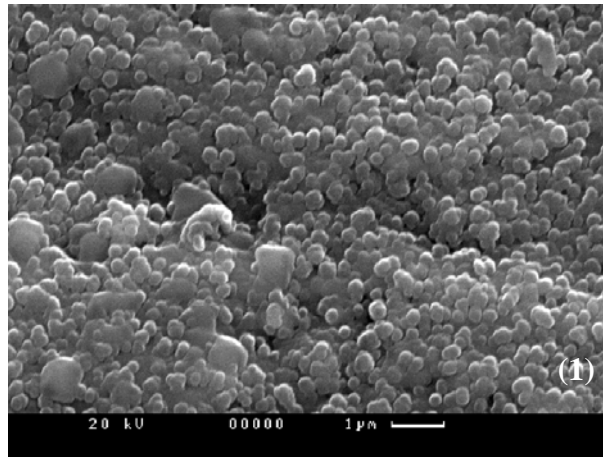


Figure A4: SEM images at 1 μm (1) and 10 μm (2) and EDS spectra (3) of HA coating on titanium with $-\text{OH}$ SAM following immersion in SBF for 5 days.

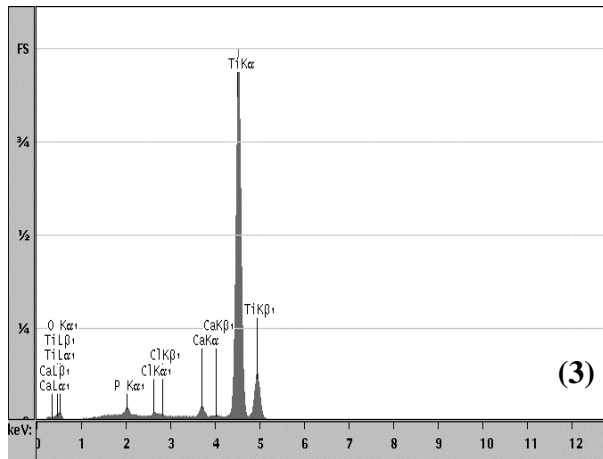
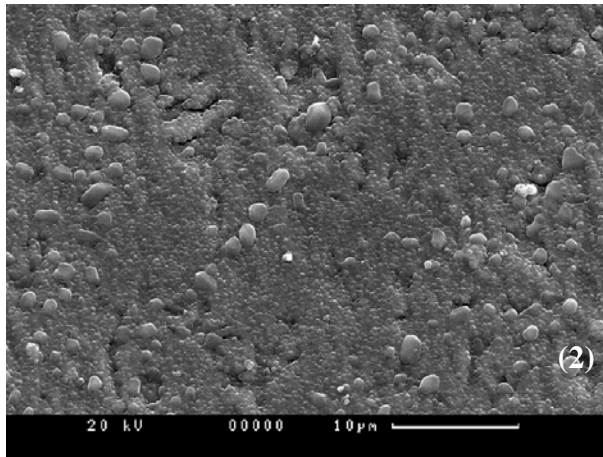
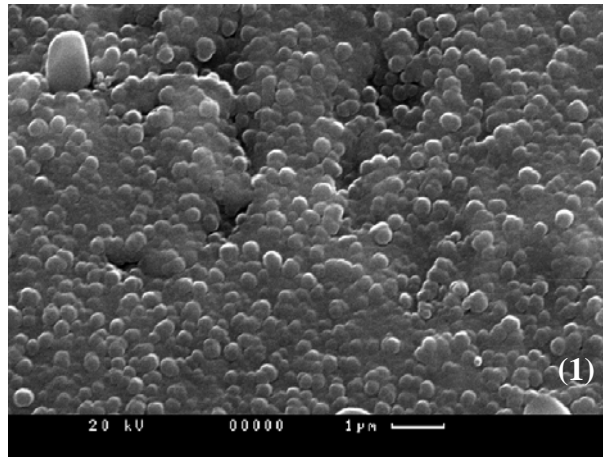


Figure A5: SEM images at 1 μm (1) and 10 μm (2) and EDS spectra (3) of HA coating on titanium with $-\text{SO}_3\text{H}$ SAM following immersion in SBF for 5 days.

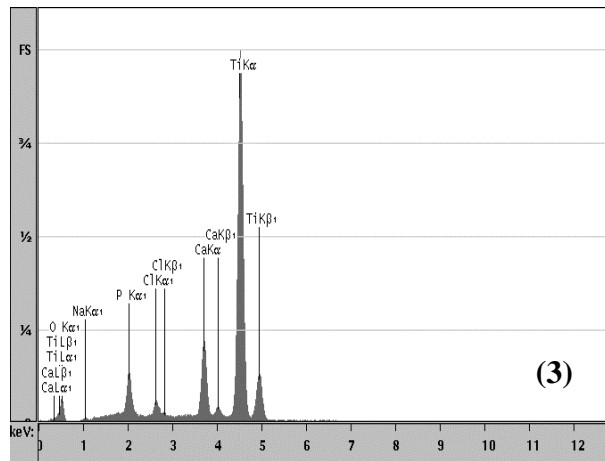
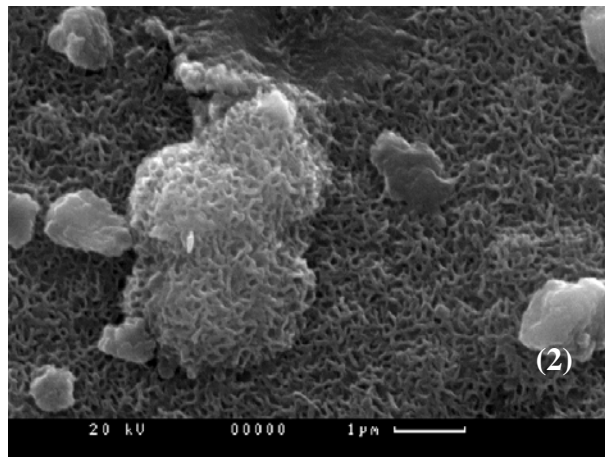
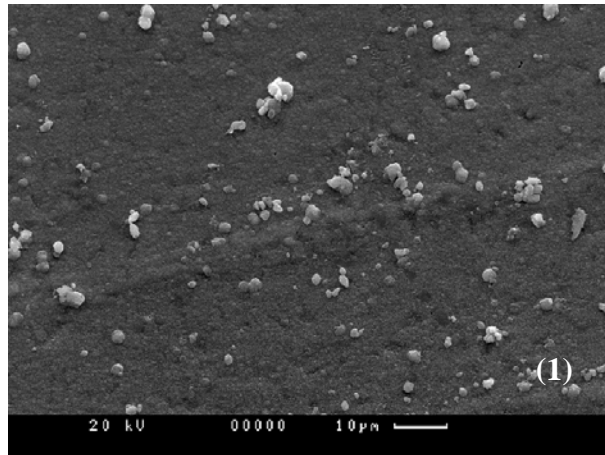


Figure A6: SEM images at 30 μm (1) and 1 μm (2) and EDS spectra (3) of HA coating on titanium with $-\text{OH}$ SAM following immersion in SBF for 30 days.

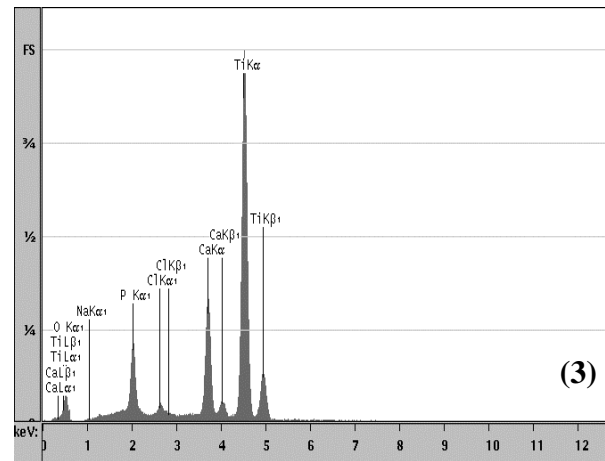
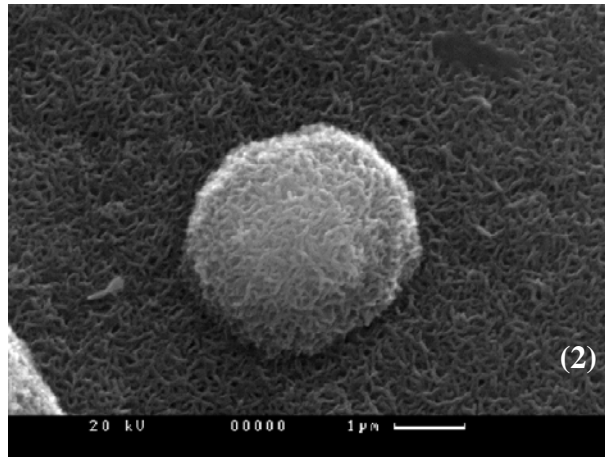
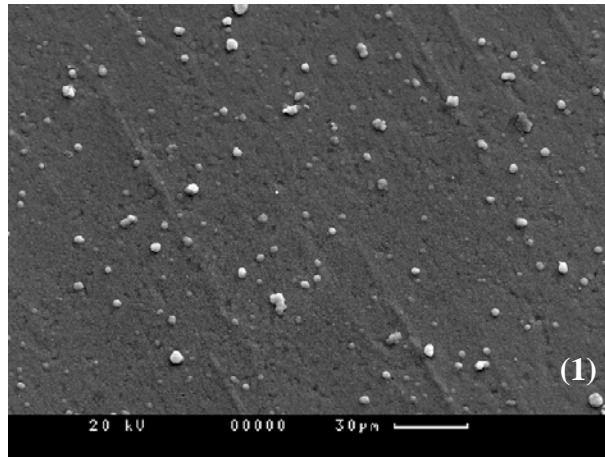


Figure A7: SEM images at 30 μm (1) and 1 μm (2) and EDS spectra (3) of HA coating on titanium with $-\text{SO}_3\text{H}$ SAM following immersion in SBF for 30 days.

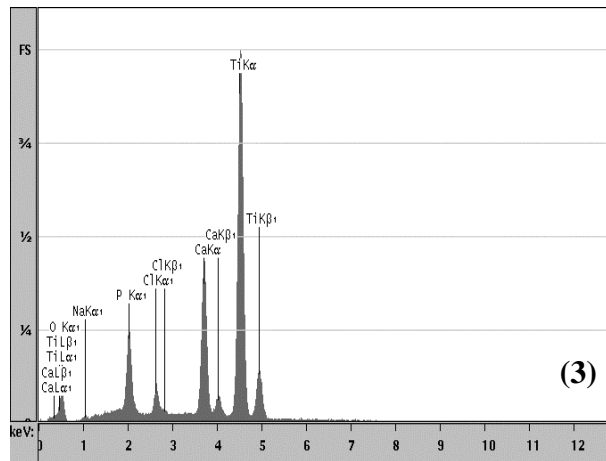
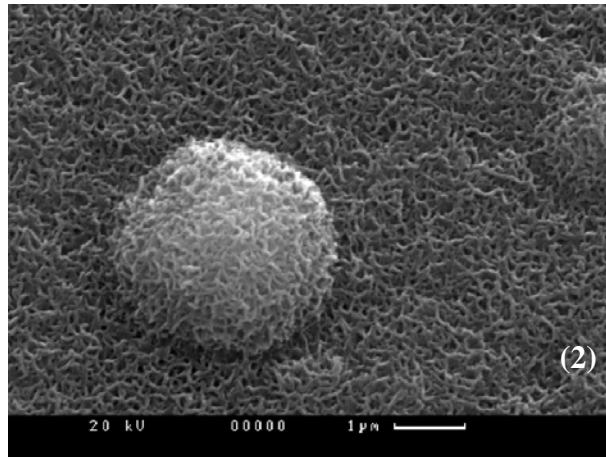
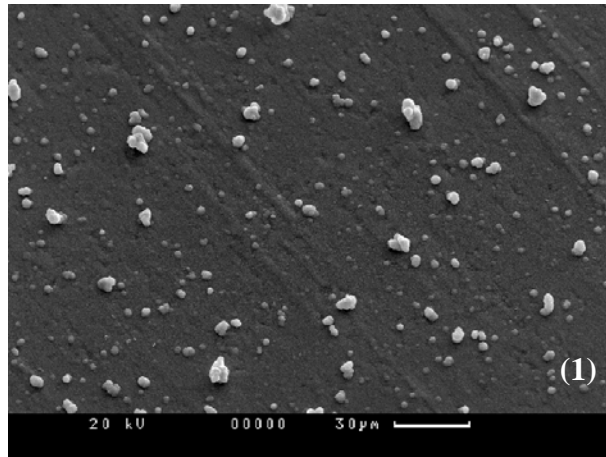


Figure A8: SEM images at 30 μm (1) and 1 μm (2) and EDS spectra (3) of HA coating on titanium with $-\text{PO}_4\text{H}_2$ SAM following immersion in SBF for 30 days.

Appendix B: Results of EDS Analysis

Table B1: EDS analysis of various samples immersed in SBF for 5 days.

| Element | Gross | Net | %Wt | %At Wt | K-Ratio | Ca/P |
|-------------------------------------|---------|---------|-------|--------|---------|------|
| Ti | | | | | | |
| P | 62.63 | 44.04 | 1.54 | 1.82 | 0.01 | 0.79 |
| Ca | 75.19 | 47.6 | 1.56 | 1.42 | 0.02 | |
| Cl | 55.36 | 32.41 | 1.11 | 1.15 | 0.01 | |
| Ti | 1735.84 | 1710.44 | 80.92 | 61.69 | 0.94 | |
| O | 30.67 | 26.83 | 14.86 | 33.92 | 0.01 | |
| -OH SAM | | | | | | |
| P | 68.03 | 47.89 | 1.54 | 1.81 | 0.01 | 0.78 |
| Ca | 83.41 | 51.23 | 1.54 | 1.4 | 0.02 | |
| Cl | 49.79 | 24.48 | 0.77 | 0.79 | 0.01 | |
| Ti | 1901.39 | 1871.71 | 81.14 | 61.78 | 0.94 | |
| O | 33.27 | 29.55 | 15.01 | 34.22 | 0.02 | |
| -SO ₃ H SAM | | | | | | |
| P | 73.8 | 47.91 | 1.57 | 1.8 | 0.01 | 0.97 |
| Ca | 95.31 | 63.96 | 1.96 | 1.74 | 0.03 | |
| Cl | 52.64 | 22.37 | 0.72 | 0.72 | 0.01 | |
| Ti | 1816.68 | 1784.04 | 79 | 58.56 | 0.93 | |
| O | 36.97 | 33.01 | 16.75 | 37.18 | 0.02 | |
| -PO ₄ H ₂ SAM | | | | | | |
| P | 76.69 | 55.83 | 1.78 | 2.05 | 0.02 | 0.84 |
| Ca | 97.33 | 64.29 | 1.93 | 1.72 | 0.03 | |
| Cl | 52.89 | 26.61 | 0.83 | 0.84 | 0.01 | |
| Ti | 1860.59 | 1828.64 | 79.04 | 58.82 | 0.93 | |
| O | 37.16 | 33.08 | 16.41 | 36.57 | 0.02 | |
| -COOH SAM | | | | | | |
| P | 79.11 | 53 | 1.81 | 2.05 | 0.02 | 1.22 |
| Ca | 114.61 | 86.77 | 2.84 | 2.48 | 0.04 | |
| Cl | 74.56 | 44.21 | 1.49 | 1.47 | 0.02 | |
| Ti | 1646.43 | 1620.88 | 75.68 | 55.27 | 0.9 | |
| Na | 22.03 | 10.8 | 1.54 | 2.34 | 0 | |
| O | 35.91 | 31.95 | 16.64 | 36.39 | 0.02 | |

Table B2: EDS analysis of various samples immersed in SBF for 30 days.

| Element | Gross | Net | %Wt | %At Wt | K-Ratio | Ca/P |
|-------------------------------------|---------|---------|-------|--------|---------|------|
| Ti | | | | | | |
| P | 213.16 | 186.2 | 4.44 | 4.45 | 0.05 | |
| Ca | 388.44 | 347.88 | 8.56 | 6.63 | 0.13 | 1.49 |
| Cl | 103.29 | 68.81 | 1.67 | 1.46 | 0.02 | |
| Ti | 1772.71 | 1731.87 | 59.92 | 38.8 | 0.77 | |
| O | 78.95 | 73.43 | 24.42 | 47.33 | 0.03 | |
| -OH SAM | | | | | | |
| P | 269.89 | 239.33 | 6.23 | 6.07 | 0.07 | |
| Ca | 477.95 | 441.37 | 12.21 | 9.2 | 0.19 | 1.52 |
| Cl | 74.29 | 40.57 | 1.1 | 0.94 | 0.01 | |
| Ti | 1426.33 | 1389.37 | 54.08 | 34.09 | 0.69 | |
| O | 79.81 | 73.69 | 26.25 | 49.53 | 0.04 | |
| -SO ₃ H SAM | | | | | | |
| P | 285.61 | 251.92 | 6.06 | 5.9 | 0.07 | |
| Ca | 547.93 | 508.43 | 12.69 | 9.54 | 0.2 | 1.62 |
| Cl | 148.11 | 110.73 | 2.77 | 2.36 | 0.03 | |
| Ti | 1466.85 | 1427.01 | 51.9 | 32.65 | 0.66 | |
| O | 86.61 | 78.33 | 25.69 | 48.38 | 0.04 | |
| -PO ₄ H ₂ SAM | | | | | | |
| P | 272.24 | 240.12 | 6.44 | 6.06 | 0.07 | |
| Ca | 526.12 | 491.48 | 12.94 | 9.4 | 0.22 | 1.55 |
| Cl | 112.24 | 75.76 | 21.29 | 1.74 | 0.03 | |
| Ti | 1258.23 | 1225 | 50.04 | 30.41 | 0.64 | |
| O | 83.71 | 77.59 | 27.81 | 50.59 | 0.04 | |
| -COOH SAM | | | | | | |
| P | 260.81 | 231.59 | 6.53 | 6.28 | 0.07 | |
| Ca | 480.67 | 448.84 | 13.61 | 10.12 | 0.21 | 1.61 |
| Cl | 80.21 | 48.25 | 1.42 | 1.19 | 0.02 | |
| Ti | 1231.87 | 1200.56 | 51.17 | 31.82 | 0.66 | |
| O | 75.29 | 70.85 | 26.94 | 50.16 | 0.04 | |

Table B3: EDS analysis from the one-half fractional factorial experiments (the first replicate).

| Replication One | | | | | | | |
|-----------------|-------|-------|---------|---------|-------|-------|---------|
| Element | Wt % | At % | K-Ratio | Element | Wt % | At % | K-Ratio |
| ad | | | | 1 | | | |
| Na | 2.2 | 4.43 | 0.0064 | Na | 2.56 | 5.11 | 0.0074 |
| Mg | 0.87 | 1.65 | 0.0037 | Mg | 1.15 | 2.16 | 0.0049 |
| P | 0.45 | 0.67 | 0.0037 | P | 0.66 | 0.98 | 0.0054 |
| Ca | 0.84 | 0.97 | 0.0103 | Ca | 0.39 | 0.44 | 0.0047 |
| Ti | 95.64 | 92.28 | 0.9481 | Ti | 95.25 | 91.3 | 0.9444 |
| Total | 100 | 100 | | Total | 100 | 100 | |
| bd | | | | ab | | | |
| Na | 2.56 | 5.05 | 0.0075 | Na | 4.57 | 8.89 | 0.0136 |
| Mg | 2.12 | 3.95 | 0.0091 | Mg | 0.71 | 1.31 | 0.003 |
| P | 1.01 | 1.48 | 0.0083 | P | 0.81 | 1.16 | 0.0066 |
| Ca | 0.7 | 0.79 | 0.0085 | Cl | 2.44 | 3.08 | 0.0236 |
| Ti | 93.62 | 88.73 | 0.9249 | Ca | 0.66 | 0.74 | 0.0079 |
| Total | 100 | 100 | | Ti | 90.81 | 84.81 | 0.8924 |
| cd | | | | Total | | | |
| Na | 7.6 | 14.26 | 0.0234 | 100 | | | |
| Mg | 0.73 | 1.3 | 0.0031 | ac | | | |
| P | 0.73 | 1.08 | 0.0063 | Na | 2.46 | 4.89 | 0.0072 |
| Cl | 4.1 | 4.99 | 0.0393 | Mg | 1.61 | 3.02 | 0.0069 |
| Ca | 1.08 | 1.16 | 0.0125 | P | 0.66 | 0.98 | 0.0055 |
| Ti | 85.71 | 77.21 | 0.8343 | Ca | 1.24 | 1.42 | 0.0152 |
| Total | 100 | 100 | | Ti | 94.03 | 89.7 | 0.9288 |
| abcd | | | | Total | | | |
| Na | 2.36 | 4.71 | 0.0069 | 100 | | | |
| Mg | 1.33 | 2.52 | 0.0057 | bc | | | |
| P | 0.94 | 1.39 | 0.0078 | Na | 2.07 | 4.12 | 0.006 |
| Ca | 0.62 | 0.71 | 0.0076 | Mg | 1.93 | 3.64 | 0.0083 |
| Ti | 94.74 | 90.68 | 0.938 | P | 0.79 | 1.16 | 0.0065 |
| Total | 100 | 100 | | Ca | 0 | 0 | 0 |
| Total | | | | Ti | 95.22 | 91.08 | 0.9444 |
| 100 | | | | Total | 100 | 100 | |

Table B4: EDS analysis from the one-half fractional factorial experiments (the second replicate).

| Replication Two | | | | | | | |
|-----------------|-------|-------|---------|---------|-------|-------|---------|
| Element | Wt % | At % | K-Ratio | Element | Wt % | At % | K-Ratio |
| ad | | | | 1 | | | |
| Na | 0 | 0 | 0 | Na | 4.34 | 8.62 | 0.0126 |
| Mg | 0 | 0 | 0 | Mg | 0 | 0 | 0 |
| P | 0.33 | 0.5 | 0.0027 | P | 0.36 | 0.53 | 0.003 |
| Ca | 0.67 | 0.8 | 0.0083 | Ca | 0 | 0 | 0 |
| Ti | 99 | 98.7 | 0.987 | Ti | 95.3 | 90.85 | 0.9464 |
| Total | 100 | 100 | | Total | 100 | 100 | |
| bd | | | | ab | | | |
| Na | 0.7 | 1.42 | 0.002 | Na | 4.41 | 8.73 | 0.0129 |
| Mg | 1.17 | 2.25 | 0.0051 | Mg | 0 | 0 | 0 |
| P | 1.58 | 2.37 | 0.0131 | P | 0.64 | 0.94 | 0.0053 |
| Ca | 0.79 | 0.91 | 0.0096 | Ca | 0.97 | 1.1 | 0.0119 |
| Ti | 95.76 | 93.05 | 0.9488 | Ti | 93.97 | 89.22 | 0.9294 |
| Total | 100 | 100 | | Total | 100 | 100 | |
| cd | | | | ac | | | |
| Na | 1.41 | 2.87 | 0.0041 | Na | 1.54 | 3.12 | 0.0044 |
| Mg | 0.65 | 1.25 | 0.0028 | Mg | 0.74 | 1.43 | 0.0032 |
| P | 0.71 | 1.07 | 0.0059 | P | 0.45 | 0.68 | 0.0037 |
| Ca | 0.99 | 1.15 | 0.0122 | Ca | 0.58 | 0.67 | 0.0072 |
| Ti | 96.23 | 93.66 | 0.9545 | Ti | 96.69 | 94.1 | 0.9605 |
| Total | 100 | 100 | | Total | 100 | 100 | |
| abcd | | | | bc | | | |
| Na | 1.41 | 2.89 | 0.004 | Na | 6.15 | 11.99 | 0.0181 |
| Mg | 0 | 0 | 0 | Mg | 0 | 0 | 0 |
| P | 0.45 | 0.69 | 0.0038 | P | 0.42 | 0.61 | 0.0067 |
| Ca | 0.26 | 0.3 | 0.0032 | Ca | 0 | 0 | 0 |
| Ti | 97.88 | 96.12 | 0.975 | Ti | 93.43 | 87.4 | 0.9232 |
| Total | 100 | 100 | | Total | 100 | 100 | |

Table B5: The one-half fractional factorial experiments results.

| Run | Process variables | | | | | Ca/P | Ca |
|-----|---------------------------|------------------------|----------------------|------------------------|--------------------------|-------------------|-----------------|
| | Ca con. (X ₁) | Ca/P (X ₂) | pH (X ₃) | Tem. (X ₄) | Blocks (X ₅) | Y _{Ca/P} | Y _{Ca} |
| 1 | 5 | 1.57 | 6.5 | 67 | 1 | 1.44 | 0.97 |
| 2 | 1.875 | 2.5 | 6.5 | 67 | 1 | 0.43 | 0.79 |
| 3 | 1.875 | 1.57 | 8.4 | 67 | 1 | 1.07 | 1.22 |
| 4 | 5 | 2.5 | 8.4 | 67 | 1 | 0.51 | 0.71 |
| 5 | 1.875 | 1.57 | 6.5 | 18 | 1 | 0.49 | 0.44 |
| 6 | 5 | 2.5 | 6.5 | 18 | 1 | 0.63 | 0.76 |
| 7 | 5 | 1.57 | 8.4 | 18 | 1 | 1.45 | 1.42 |
| 8 | 1.875 | 2.5 | 8.4 | 18 | 1 | 0 | 0 |
| 9 | 5 | 1.57 | 6.5 | 67 | 2 | 1.6 | 0.8 |
| 10 | 1.875 | 2.5 | 6.5 | 67 | 2 | 0.38 | 0.91 |
| 11 | 1.875 | 1.57 | 8.4 | 67 | 2 | 1.07 | 1.15 |
| 12 | 5 | 2.5 | 8.4 | 67 | 2 | 0.43 | 0.3 |
| 13 | 1.875 | 1.57 | 6.5 | 18 | 2 | 0.47 | 0.25 |
| 14 | 5 | 2.5 | 6.5 | 18 | 2 | 1.17 | 1.1 |
| 15 | 5 | 1.57 | 8.4 | 18 | 2 | 0.99 | 0.67 |
| 16 | 1.875 | 2.5 | 8.4 | 18 | 2 | 0 | 0 |

Appendix C: Results of Osteoblast Cells Culture Experiment

Table C1: Absorption values at 570 nm on coated and uncoated Ti substrates following culturing for 4 and 6 weeks.

| Day | Normal Human Osteoblasts | | | |
|-----------|--------------------------|----------|----------|----------|
| | donor-ML | | donor-MY | |
| | Coated | Uncoated | Coated | Uncoated |
| 28 | 0.688 | 0.115 | 0.791 | 0.374 |
| | 0.834 | 0.22 | 0.801 | 0.278 |
| | 0.753 | 0.181 | 0.278 | 0.529 |
| | Average | 0.758333 | 0.172 | 0.623333 |
| 42 | 0.881 | 0.402 | 0.789 | 0.712 |
| | 0.547 | 0.416 | 0.756 | 0.684 |
| | 0.786 | 0.408 | 0.737 | 0.36 |
| | Average | 0.641667 | 0.408667 | 0.760667 |

Table C2: Calcium concentrations on coated and uncoated Ti substrates following culturing for 4 and 6 weeks.

| Day | Normal Human Osteoblasts | | | |
|-----------|--------------------------|----------|----------|----------|
| | donor-ML | | donor-MY | |
| | Coated | Uncoated | Coated | Uncoated |
| 28 | 1.428 | 0 | 1.690 | 0.631 |
| | 1.799 | 0.240 | 1.715 | 0.387 |
| | 1.593 | 0.141 | 0.387 | 1.024 |
| | Average | 1.607 | 0.127 | 1.264 |
| 42 | 3.879 | 0.065 | 3.146 | 2.533 |
| | 1.220 | 0.177 | 2.884 | 2.311 |
| | 3.123 | 0.113 | 2.732 | 0 |
| | Average | 2.740 | 0.118 | 2.921 |

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