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# APPENDICES

## Appendix A Ninhydrin Analysis

**Table A1** The absorbance of 1,6-hexanediamine in 1,4-dioxane-isopropanol (1:1, v/v) of known concentration solution

| Std No. | Std. HMD concentration | Average Absorbance |
|---|---|---|
| 1 | 0.00002 | 0.0086 |
| 2 | 0.00004 | 0.0223 |
| 3 | 0.00006 | 0.0354 |
| 4 | 0.00010 | 0.0616 |
| 5 | 0.00020 | 0.1232 |
| 6 | 0.00040 | 0.2707 |

**Figure A1** The calibration curve obtained with 1,4-dioxane-isopropanol (1:1, v/v) solution containing 1,6-hexanediamine of known concentration.

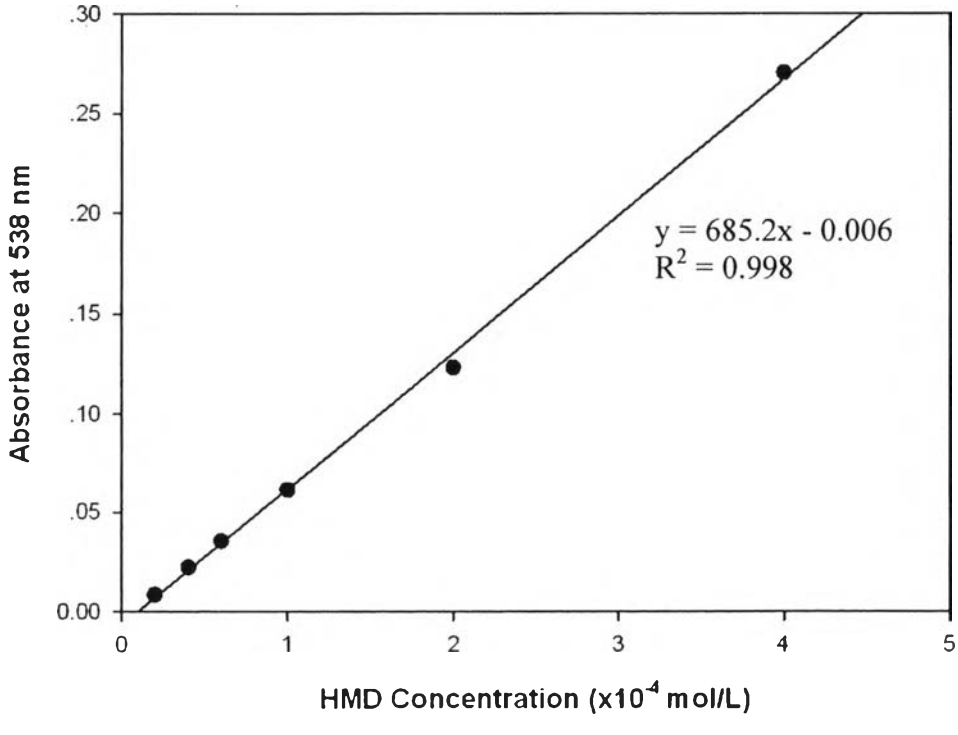


**Table A2** $NH_2$ density as a function of 1,6-hexanediamine concentration

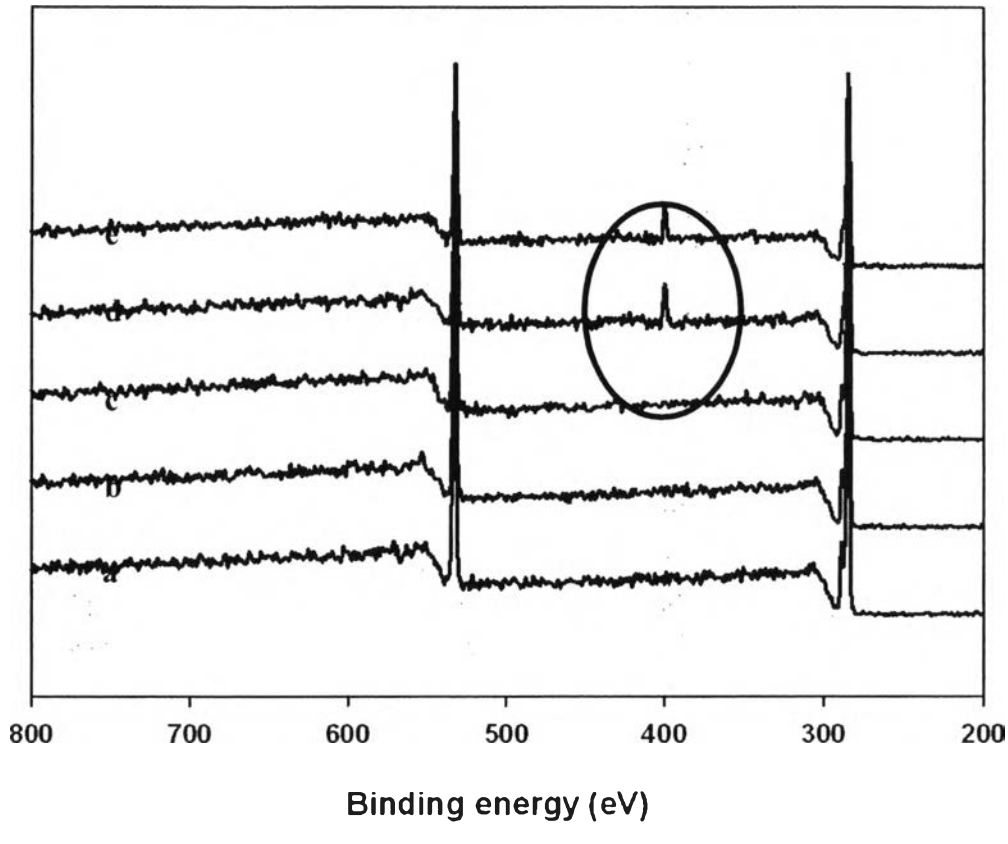
| **Diamine concentration (mol/L)** | **$NH_2$ conc. ($x10^{-8}$) mol/cm$^2$** |
|---|---|
| 0.5 | 2.93 ± 0.05 |
| 1.0 | 3.40 ± 0.02 |
| 1.5 | 3.68 ± 0.02 |
| 2.0 | 4.45 ± 0.01 |

**Table A3** $NH_2$ density as a function of aminolyzing time

| **Aminolyzing time (h)** | **$NH_2$ conc. ($x10^{-7}$) mol/cm$^2$** |
|---|---|
| 2 | 6.41 ± 0.01 |
| 4 | 7.34 ± 0.02 |
| 6 | 8.33 ± 0.01 |
| 8 | 31.86 ± 0.02 |
| 10 | 13.58 ± 0.02 |

## Appendix B X-ray Photoelectron Spectrometer (XPS)

**Figure B1** The survey XPS spectra of (a) neat PCL, (b) PCL aminolyzed in 1.5 M HMD/IPA solution for 8 h at room temperature, (c) activatedPCL, (d) PCL immobilized with crude bone protein (3 mg/ml), and (e) PCL immobilized with bovine serum albumin (3 mg/ml).



## Appendix C Experimental Data of biological characterizations

**Table C1** Raw data of indirect cytotoxicity test of all types of PCL film mats, determined the viability of cells by MTT assay method at 570 nm

| Time (day) | % viability of MC3T3-E1 cells (relative to TCPs at 1 day) | | | |
|---|---|---|---|---|
| | 1 | | 7 | |
| **Material** | **avg** | **SD** | **avg** | **SD** |
| TCPs (control) | 100.00 | 0.55 | 100 | 0.75 |
| neat PCL | 99.28 | 0.96 | 104.62 | 1.02 |
| aminolyzed PCL | 98.00 | 1.07 | 102.04 | 0.20 |
| CBP – immobilized PCL | 102.12 | 0.93 | 102.63 | 0.46 |
| BSA - immobilized PCL | 102.47 | 0.40 | 106.89 | 3.18 |

**Table C2** Raw data of cell attachment of MC3T3-E1 onto all types of PCL film mats at 6 and 24 h, determined the viability of cells by MTT assay method at 570 nm

| Time (h) | % viability of MC3T3-E1 cells (relative to TCPs at 24 h) | | | |
|---|---|---|---|---|
| | 6 | | 24 | |
| **Material** | **avg** | **SD** | **avg** | **SD** |
| TCPs (control) | 61.52 | 0.55 | 100.00 | 0.65 |
| neat PCL | 25.09 | 0.79 | 62.45 | 1.39 |
| aminolyzed PCL | 29.37 | 0.40 | 68.22 | 1.91 |
| CBP – immobilized PCL | 35.13 | 1.35 | 80.67 | 0.49 |
| BSA - immobilized PCL | 34.94 | 0.67 | 95.72 | 2.41 |

**Table C3** Raw data of cell proliferation of MC3T3-E1 onto all types of PCL film mats at 1, 2, and 3 d, determined the viability of cells by MTT assay method at 570 nm

| time(day) | % viability of MC3T3-E1 cells (relative to TCPs at 1 day) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | |
| **Material** | **avg** | **SD** | **avg** | **SD** | **avg** | **SD** |
| TCPs (control) | 100.00 | 0.65 | 151.67 | 1.27 | 387.92 | 3.22 |
| neat PCL | 62.45 | 1.39 | 107.06 | 0.66 | 194.24 | 5.58 |
| aminolyzed PCL | 109.23 | 1.91 | 112.45 | 3.90 | 227.13 | 1.34 |
| CBP – immobilized PCL | 118.26 | 0.49 | 112.82 | 3.23 | 270.073 | 1.62 |
| BSA - immobilized PCL | 118.66 | 2.41 | 147.21 | 3.53 | 464.31 | 1.72 |

**Figure C1** ALP Assay of MC3T3-E1 seeded onto all types of PCL film mats at 3, 5 and 7 days.

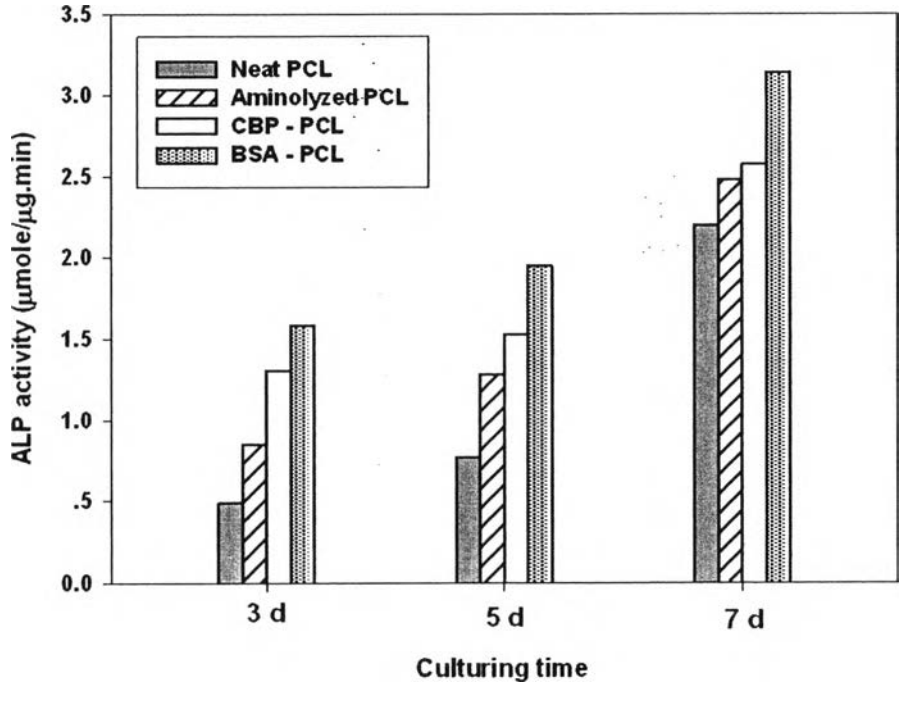


**Figure C2** Protein Assay of MC3T3-E1 seeded onto all types of PCL film mats at 3, 5 and 7 days.

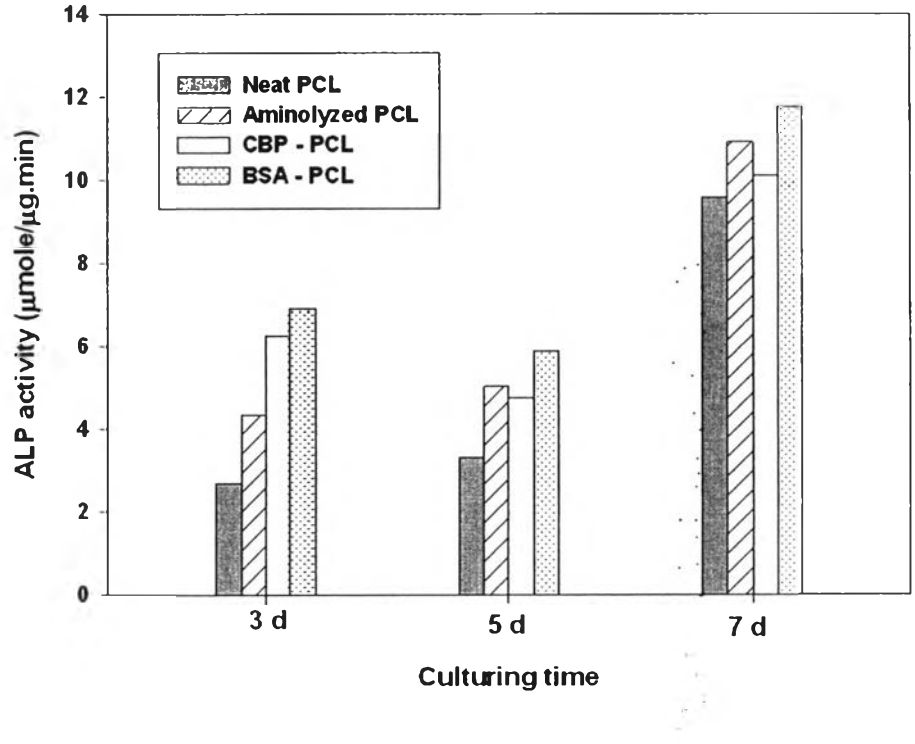



**Table C4** Raw data of quantity of mineral deposition on all types of PCL film mats after cell culturing for 21 d using Alizarin Red-S method at 570 nm

| Materials | 1 | 2 | avg | SD |
|---|---|---|---|---|
| TCPs (control) | 0.275 | 0.262 | 0.269 | 0.009 |
| neat PCL | 0.516 | 0.432 | 0.474 | 0.059 |
| aminolyzed PCL | 0.836 | 0.927 | 0.8815 | 0.064 |
| CBP – immobilized PCL | 1.862 | 1.865 | 1.8635 | 0.002 |
| BSA - immobilized PCL | 3.100 | 3.044 | 3.072 | 0.039 |

# CURRICULUM VITAE

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**Presentations:**

1. Satianyanond, S.; Pavasant, P.; and Supaphol, P. (2011, April 26) Surface Modification of Polycaprolactone Membrane via Aminolysis and Protein-Immobilization for Promoting Bone Cell Growth. Poster presented at the 17th PPC Symposium on Petroleum, Petrochems, and Polymers, Bangkok, Thailand.