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Published on Web 02/18/2009

A Nanoscale Adhesion Layer to Promote Cell Attachment on PEEK

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Received December 26, 2008; E-mail: jschwart@princeton.edu

Poly(aryl-ether-ether-ketone) (PEEK) is emerging as an important biomaterial for use in trauma, orthopedic, and spine applications.[1] It has distinct advantages over metallic implant materials in that it can be combined with carbon fiber to have mechanical properties that more nearly replicate those of bone, and it is radiolucent, which facilitates radiographical analysis of tissue surrounding implanted devices. Paradoxically, it suffers compared to metals in that the PEEK surface is quite bioinert, so much so that even desired cell growth on it is severely limited. Indeed, it is this low ability of PEEK to foster surface cell growth that has limited its application to devices where tissue fixation is critical. To this end, considerable effort has been made to create composites of PEEK and hydroxyapatite (HA) in attempts to combine the beneficial mechanical properties of the plastic with possible osseoconductive properties of HA.[1] Unfortunately, these efforts have resulted in trade-offs between desirable cell surface and materials mechanical properties. Common chemical surface treatments have also failed to yield materials displaying both beneficial mechanical and cell growth properties. We have reported that polymers with functional groups that can be acidified, such as N−H groups of polyamides[2] or polyurethanes,[3] can be surface activated for cell adhesion by treatment with vapor of zirconium or titanium alkoxides followed by ligand replacement reactions with organics that can be used to bond cell adhesive peptides. This process is not, however, applicable to PEEK, which is simply a polyether polyketone.[1] We now report that an adhesion layer can be prepared on PEEK from these alkoxides in a process reminiscent of deposition and partial thermolysis of metal alkoxides on oxide surfaces:[4] a thin film of the alkoxide is vapor deposited onto the PEEK surface, and the metal complex is bound through simple ligation. Controlled thermolysis of the alkoxide coating gives a mixed alkoxide−oxide "adhesion layer," which is then used to attach organophosphonates or carboxylates through methods comparable to those we have described for native metal oxide surfaces.[5]

We had observed that deposition of zirconium tetra(*tert*-butoxide) (**1**) onto metal oxide surfaces followed by mild thermal treatment (ca. 50 °C) in UHV gave a surface bound Zr species with stoichiometry consistent with a mixed alkoxide−oxide.[4] We hypothesized that carbonyl or ether groups at the surface of polyesters, polyethers, or polyketones could similarly serve as coordinating groups to enable deposition of a metal alkoxide complex onto the polymer surface, and that mild thermolysis would give an analogous mixed metal alkoxide−oxide layer that would be bound to the polymer through coordinative interactions between these surface ether or carbonyl groups and the metal centers.

In typical experiments coupons of PEEK (Goodfellow) were treated with the vapor of **1** or titanium tetra(*tert*-butoxide) (**2**) at 10^{-3} Torr with external evacuation for 30 s followed by 5 min exposure without external evacuation. They were next heated to 75 °C and then sonicated for 1 min in dry acetonitrile to give **3a**. A similar sequence done on a 0.5 mm thick film of poly(ethylene terephthalate) (PET), but with sonication in THF, gave **3b**, which was imaged by atomic force microscopy (AFM). IR spectra of **3a** and **3b** showed $\nu_{C-H} = 2976$ cm^{-1}, indicative of *tert*-butoxide groups.[6] The static water contact angle (90°) measured for **3a** decreased to 35° as the *tert*-butoxy ligands were cleaved by exposure to ambient water. Samples of **3a** and **3b** were treated with octadecylphosphonic acid (ODPA) via the T-BAG method,[7] which gave phosphonate adducts **4a** and **4b** (water contact angle = 95°; IR, $\nu_{CH2,asym} = 2920$ cm^{-1}; $\nu_{CH2,sym} = 2849$ cm^{-1}, characteristic of disordered alkyl chains[8]). X-ray photoelectron spectroscopy of coated **4a** showed Zr (3d) and P (2p) peaks with Zr/P ≅ 2:1 (see Supporting Information), consistent with a model in which the Zr alkoxide−oxide forms a bilayer and only the topmost layer reacts with the phosphonic acid (Scheme 1). A sample of **4b** was vigorously flexed and wiped with a Kimwipe. AFM analysis of the resulting material showed a film thickness of 3−4 nm (determined from pin holes in the film; Figure 1); since the phosphonate is about 2 nm long, this suggests that the adhesion layer is 1−2 nm thick, consistent with the XPS data.

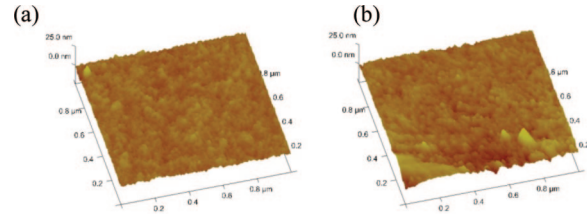


Figure 1. AFM images of (a) PET and (b) **3b** on PET.

The relationship between deposition and heating times of **1** and adhesion layer thickness was probed via quartz crystal microgravimetry (QCM) using a silicon QCM crystal surrogate placed in the deposition chamber alongside samples of PET and PEEK. The change in the crystal frequency following deposition (at 10^{-3} Torr) and heating (at ca. 50 °C) is related to the mass of the adhesion layer (**3c**) that has been deposited on the crystal.[5,9] Layer thicknesses were estimated assuming that the adhesion layer packs with a density similar to that of zirconia and were calculated as the quotient of the measured aerial surface density of **3c** on the QCM crystal (in ng/cm^2) and the known density of zirconia (5.89×10^9 ng/cm^3) (Table 1).

PEEK was cut into 1.125″ × 0.5″ coupons that were treated with **1** to give **3a** and then glued with Cytec Fiberite FM 1000

Table 1. Deposition, Heating Times, And Thickness of **3c**

deposition time	heating time	ΔQCM frequency	approx. layer thickness
5 min	10 min	296 ± 8 hz	1 nm
10 min	20 min	597 ± 11 hz	2 nm
1 h	1 h	1966 ± 33 hz	8 nm

10.1021/ja810075c CCC: $40.75

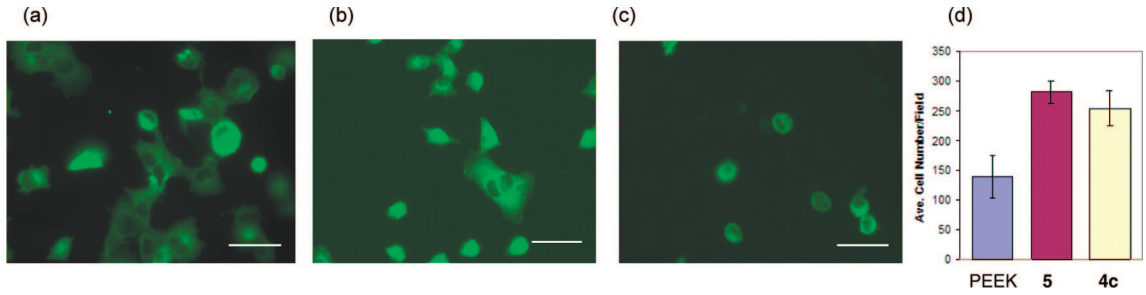
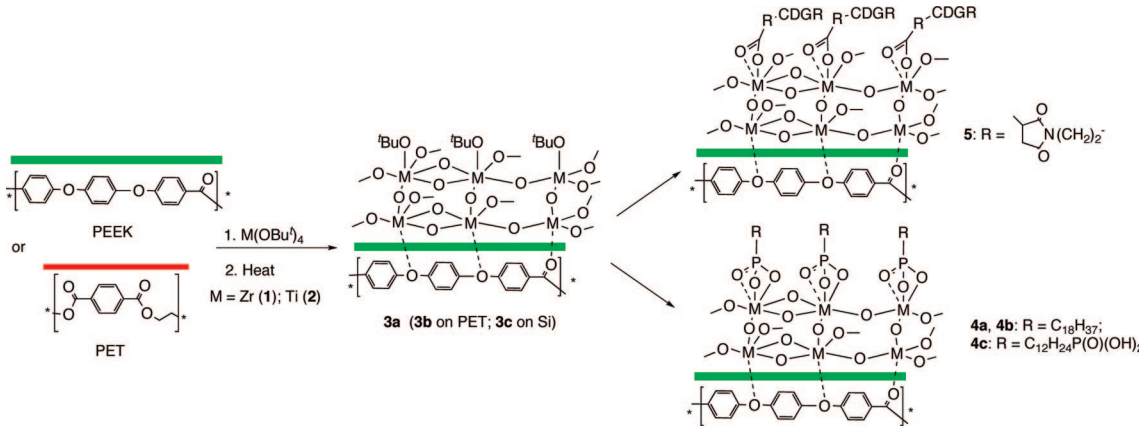


Figure 2. Osteoblast cell attachment on derivatized PEEK. (a) Cells on RGD-modified PEEK (**5**), (b) 1,12-dodecylbisphosphonate-modified PEEK (**4c**), or (c) PEEK control surfaces, all after 3 h, fixed and stained with antivinculin antibodies and fluorescein-conjugated secondary antibodies. Scale bars are 50 μm. (d) Number of cells per 10× microscope field counted for untreated PEEK, RGD-derivatized, and 1,12-dodecylbisphosphonate-derivatized PEEK. Average values from at least three fields are shown with error bars representing ±1 standard deviation.

Scheme 1. Deposition of Titanium or Zirconium Tetra(*tert*-butoxide) onto PEEK, PET, or Si Followed by Heating Gives the Adhesion Layer **3**, Which May Be Converted to a Phosphonate (**4**) or a Carboxylate (**5**) Derivative by Reaction with the Corresponding Acid



epoxy to similarly sized coupons of Ti-6Al-4V. Shear force was exerted parallel to the interface of the glued coupons by an Instron Model 1331 load testing machine until breaking.[10,11] In this manner the interfacial shear strength for the adhesion layer of **3a** on PEEK was measured to be 7.8 ± 0.2 MPa, compared with 3.0 ± 0.2 MPa for an untreated PEEK coupon; the shear strength of **3a** is close to the current standard for a hydroxyapatite coating of Ti (ca. 10 MPa[12]). The adhesion layer was also shown to be stable on PEEK for at least 3 days at pH 7.5 (see Supporting Information).[2,11]

PEEK coupons of **3a** were placed in a dry solution of 3-maleimidopropionic acid in acetonitrile (0.1 mM) followed by Michael addition of RGDC to give **5**. Other coupons of **3a** were treated with 1,12-dodecylbisphosphonic acid by the T-BAG[7] method to give **4c**. *In vitro* experiments with osteoblast cells were conducted on untreated PEEK, **5**, and **4c**. Both **5** and **4c** showed significantly increased osteoblast adhesion after 3 h versus the PEEK control (one-way ANOVA: $p = 2.3 \times 10^{-4}$ and 6.3×10^{-4}, respectively); both also supported greater osteoblast spreading when compared to a PEEK control (Figure 2).

We have shown that PEEK can be rapidly activated by surface-bonding a nanoscale, oxide-based adhesion layer to it. We have also shown that subsequent bonding of organics to this adhesion layer can be easily accomplished to give surfaces that are significantly more active for cell attachment and spreading than untreated PEEK. These results suggest that the simple sequence of surface chemical treatment described can provide a new avenue for development of PEEK as a material with enhanced biomedical application.

Acknowledgment. The authors thank the National Science Foundation (CHE-0612572) for financial support, Manish Dubey for XPS spectra, Nima Rahbar for help with mechanical testing, and Prof. Jean Schwarzbauer for critical commentary.

Supporting Information Available: Full experimental details for formation of the metal alkoxide/oxide adhesion layer; XPS spectra; adhesion layer stability measurements; and cell counting and spreading protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kurtz, A. M.; Devine, J. N. *Biomaterials* **2007**, *28*, 4845–4869.
(2) Dennes, T. J.; Hunt, G. C.; Schwarzbauer, J. E.; Schwartz, J. *J. Am. Chem. Soc.* **2007**, *129*, 93–97.
(3) Dennes, T. J.; Schwartz, J. *Soft Matter* **2008**, *4*, 86–89.
(4) Miller, J. B.; Bernasek, S. L.; Schwartz, J. *J. Am. Chem. Soc.* **1995**, *117*, 4037–4041.
(5) Gawalt, E. S.; Avaltroni, M. J.; Danahy, M. P.; Silverman, B. M.; Hanson, E. L.; Midwood, K. S.; Schwarzbauer, J. E.; Schwartz, J. *Langmuir* **2003**, *19*, 200–204 (correction, *Langmuir* **2003**, *19*, 7147).
(6) Lu, G.; Purvis, K. L.; Schwartz, J.; Bernasek, S. *Langmuir* **1997**, *13*, 5791–5793.
(7) Hanson, E. L.; Schwartz, J.; Nickel, B.; Koch, N.; Danisman, M. F. *J. Am. Chem. Soc.* **2003**, *126*, 16074–16080.
(8) Sondag, A. H. M.; Raas, M. C. *J. Chem. Phys.* **1989**, *91*, 4926–4931.
(9) Sauerbrey, G. *Z. Phys.* **1959**, *155*, 206.
(10) Schwartz, J.; Avaltroni, M. J.; Danahy, M. P.; Silverman, B. M.; Hanson, E. L.; Schwarzbauer, J. E.; Midwood, K. S.; Gawalt, E. S. *Mater. Sci. Eng. C* **2003**, *23*, 395–400.
(11) Danahy, M. P.; Avaltroni, M. J.; Midwood, K. S.; Schwarzbauer, J. E.; Schwartz, J. *Langmuir* **2004**, *20*, 5333–5337.
(12) U.S. Department of Heath and Human Services, F.a.D.A., Center for Devices and Radiological Health, Ed. 2000.

JA810075C