Exposed Hydroxyapatite Particles on the Surface of Photo-crosslinked Nanocomposites and Promoted MC3T3 Cell Function

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Statement of Purpose: Biodegradable polymer nanocomposite scaffolds incorporating hydroxyapatite (HA), the major inorganic component of bone mineral, are promising in bone regeneration. Among many polymer matrices, photo-crosslinkable poly(ε-caprolactone fumarate)s and poly(propylene fumarate) have been used to form nanocomposites with HA to enhance mechanical properties and cellular functions[1,2]. However, the amount of HA that can actually appear on the surface of a composite to modify the surface properties and to interact with bone cells is still largely unknown. We have developed two series of photo-crosslinked poly(ε-caprolactone) diacrylate (PCLDA)/HA nanocomposites using PCLDA530 and 2000 (Figure 1), which were synthesized from PCL diols with molecular weights of 530 and 2000, respectively. Comparison between the original and cut disks has been made to investigate the amount of HA on the composite surface. Semi-crystalline PCLDA2000/HA nanocomposites showed significantly more exposed HA after cutting while less HA was buried in amorphous PCLDA530/HA nanocomposites. Mouse MC3T3-E1 cell proliferation and differentiation have been enhanced significantly with increasing the HA composition through improved hydrophilicity and enhanced surface stiffness.

Methods: PCLDA530 and 2000 had M_n of 1120, 3510 g.mol^{-1} and M_w of 1390, 5150 g.mol^{-1}, respectively[3]. HA (Berkeley Advanced Biomaterials) was mixed with PCLDA in CH_2Cl_2 at weight compositions of 0, 10, 20, and 30% to form a slurry and then photo-crosslinked[3]. PCLDA/HA nanocomposite disks were cut using a blade to compare with the original disks.

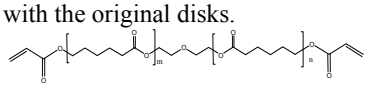

Figure 1. Chemical structure of PCLDA.

Results: As shown in Figure 2a, the surface contents of HA on crosslinked PCLDA/HA nanocomposites measured using Energy Dispersive Spectroscopy (EDS) were much lower than the feed compositions, especially for crystalline PCLDA2000/HA nanocomposites. Cut PCLDA/HA nanocomposites had HA compositions closer to the feed values and thus improved hydrophilicity was achieved as indicated by the gradual decrease in water contact angle (Figure 2b). Because of enhanced substrate stiffness and more exposed HA particles, MC3T3 cells could proliferate faster and differentiate more significantly on crosslinked PCLDA/HA nanocomposites with increasing HA composition (Figure 2c,d). For crosslinked PCLDA530/HA nanocomposites, no significant difference was found between original and cut disks. In contrast, cut semi-crystalline PCLDA2000/HA nanocomposites showed significantly better MC3T3 cell proliferation and differentiation. It demonstrated that cutting was efficient in exposing buried HA particles in crystallization and consequently promoting bone cell functions. MC3T3 cell morphology at days 1 and 4 demonstrated in Figure 2e and consistent with the cell numbers in Figure 2c.

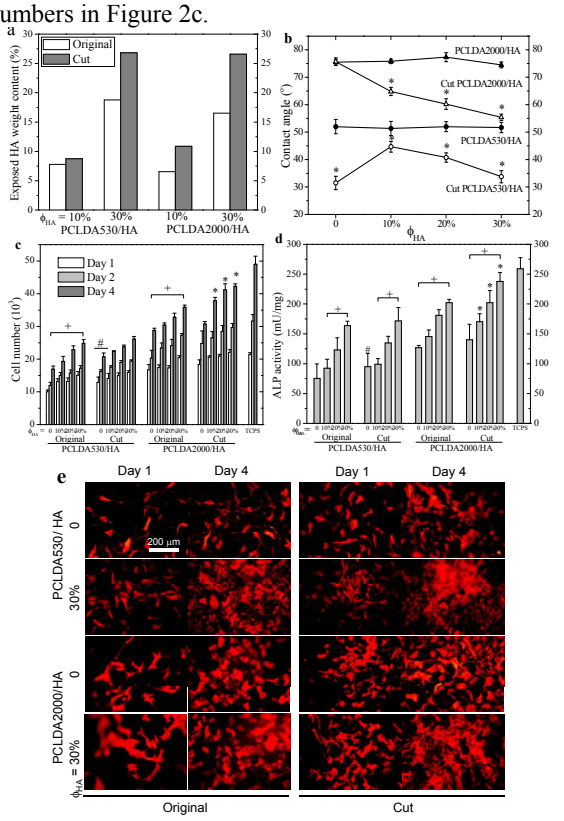

Figure 2. (a) Weight percentage of exposed HA and (b) contact angle of water on the surfaces of original and cut PCLDA/HA nanocomposites. (c) MC3T3 cell numbers at days 1, 2, and 4, (d) ALP activity at day 7, and (e) cell images at days 1 and 4 on original and cut crosslinked PCLDA/HA nanocomposites, compared with cell-seeded TCPS as positive control. Scale bar of 200 μm is applicable to all images in (e). *, $p < 0.05$ compared to data on original PCLDA/HA disks of same HA compositions at day 4. +, $p < 0.05$ between each two samples in the group at day 4. #, $p < 0.05$ compared to original crosslinked PCLDA530.

Conclusions: Photo-crosslinked amorphous PCLDA530/HA and semi-crystalline PCLDA2000/HA nanocomposites have been prepared to investigate the role of exposed HA particles on the surface in influencing MC3T3 cell behavior. We found that HA particles could be buried underneath polymer surface, especially in crystalline PCLDA2000/HA nanocomposites. Cutting is demonstrated to expose buried HA particles on the composite surface, which could significantly enhance MC3T3 cell proliferation and differentiation.

References: 1. Wang, S. *Biomaterials* **2009**, *30*, 3359.
2. Lee, K. W. *Biomaterials* **2008**, *29*, 2839.
3. Cai, L. *Polymer* **2010**, *51*, 164.

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