Promoting bioactive hydroxyapatite nucleation on modified polyurethane by adopting glycerol phosphate

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Statements of Purpose: Polyurethane (PU) based biopolymers have been widely used in various medical fields; wound dressing, cartilage replacement, bone substitutes, and artificial valves due to a highly porous structure and controllable mechanical properties [1]. However, Low biocompatibility compared to natural polymer was a limitation of PU. In order to improve the biological properties, hydroxyapatite which is well known bioceramic, was adopted with high affinity, modifying PU with glycerol phosphate (gp).

Methods: PU-gp-HA was fabricated, following 2 step; (1) glycerol phosphate conjugation and (2) hydroxyapatite mineralization. PU-gp was fabricated by mixing water based solution which involves glycerol phosphate disodium salt hydrate with TDI based PU prepolymer. PU-gp was immersed in concentrated SBF solution where predetermined amounts of 5 chemicals (sodium chloride, potassium chloride, calcium chloride, magnesium chloride and sodium phosphate monobasic) were dissolved in [2]. Mineralization was induced by adding sodium bicarbonate which modified pH of the solution from 4.4 to 6.5. Finally, PU-gp-HA was successfully formed after 1h, 4h, and 12h precipitation time. Characterization of PU-gp and PU-gp-HA was performed using P-NMR and SEM respectively. Enhanced composite stability and biological properties were also demonstrated by sonication test and *in vitro* cell test separately.

Results and discussion: First of all, glycerol phosphate was conjugated through urethane bonding with PU, where diisocyanate groups (N=C=O) of PU prepolymer reacted with glycerol phosphate. Molecular structure of PU-gp was demonstrated using P-NMR analysis (**Fig. 1**). Phosphate monoester of PU-gp induced chemical shift (~5 ppm) from standard phosphoric acid. Phosphate functional groups acted as nucleation sites for amorphous calcium phosphate in SBF solution to be anchored. Calcium phosphates were accumulated as a form of microsphere in early stage of mineralization and crystallized into HA film over PU surfaces in the late stage (**Fig. 1**). Phosphate groups not only promoted HA nucleation, but also provided robust interface between PU and HA from exterior impacts. The weight ratio of HA/PU was measured before and after sonication as well as morphology of HA after sonication (**Fig. 2**). Most HA was detached from pure PU while PU-gp maintained HA at a comparable level of w/o sonication. Filopodia of fibroblasts were well spread on PU-gp and PU-gp-HA than pure PU due to hydrophilic phosphate groups and bioactive HA. HA also activated fibroblasts' proliferation remarkably from 3 to 5 day of culturing time (**Fig. 3**).

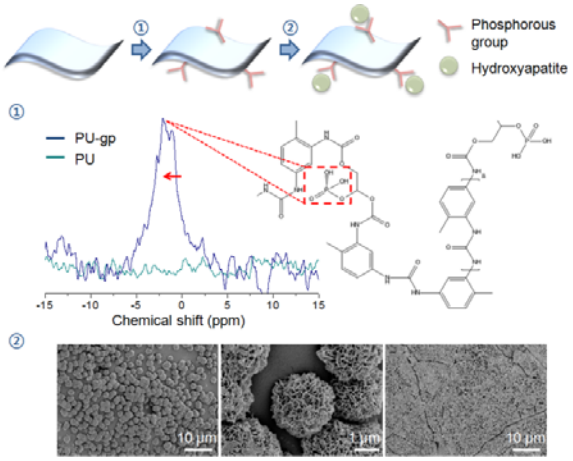

Figure 1. Schemes of 2-step PU-gp-HA fabrication; (1) characterization of glycerol phosphate conjugation by P-NMR and (2) configuration of HA using SEM

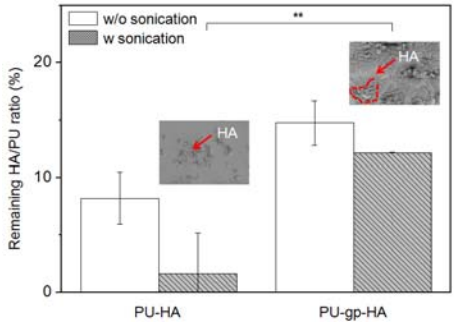

Figure 2. Stability test result according to existence of glycerol phosphate

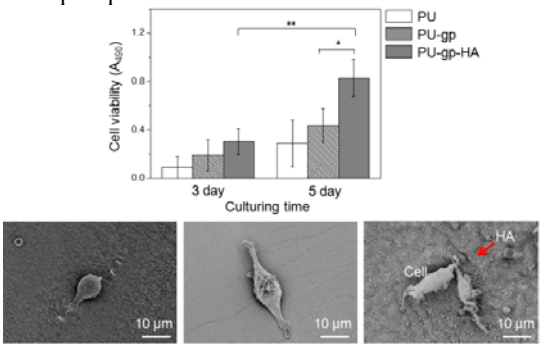

Figure 3. *In vitro* fibroblasts attachment (1 day) and viability (MTS, 3 and 5 day)

Conclusions: Glycerol phosphate was successfully conjugated on PU surfaces. It promoted HA nucleation and enhanced stability of HA on PU. Potential for PU-gp-HA to be used in various tissue engineering applications is pronouncing.

References:

[1] RJ Zdrahala et al., Journal of biomaterials applications 14(1999)67-90
[2] A.C. Tas et al., Journal of Materials Research 19(09) (2011)2742-2749