

Optimization of Phospholipid-modified Metallic Surface Relative to Initial Osteogenic Responses

R. K. Satsangi^{(1)*}, Arpan Satsangi^(1,3), J. L. Ong⁽²⁾, Neera Satsangi⁽³⁾

- (1) RANN Research Corporation, 7795 Mainland Suite 103, San Antonio, TX 78250, U.S. e-mail: rsatsangi@gmail.com
 - (2) Department of Biomedical Engineering, University of Texas at San Antonio, San Antonio, TX 78249, U.S. e-mail: ansonong@utsa.edu
 - (3) Department of Restorative Dentistry (Division of Biomaterials), University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, U.S. e-mail: satsangi@uthscsa.edu
- * Corresponding author.

Abstract – We have found that, among natural phospholipids, Phosphatidylserine (PS) is the most preferred for osteogenic induction when used for surface modification of implant materials. This effort further optimizes PS structure at its positions-1 & -2 for the same. Calcium phosphate complex of PS with varying length fatty acyls and unsaturations were coated on Ti discs and osteoblast progenitors were cultured for their initial osteogenic activities and biomineralization. PS with unsaturation in fatty-acyls performed the best. The Fetal Rat Calvarial (FRC) cells were cultured on the most active sample and the induction of biomineralization was confirmed.

1, 2- Diacyl phosphatidylserines possess lengthy fatty acyls with varying degree of unsaturation. To ascertain the role of these variants on osteogenic activities, the calcium-phosphate-complex of following PSs were made and compared for osteogenic activities when coated on Titanium (Ti) discs:

- (a) 1,2-Dipalmitoyl-sn-glycero-3-phosphatidyl-L-serine (DPPS);
- (b) 1,2-Distearoyl-sn-glycero-3-phosphatidyl-L-serine (DSPS);
- (c) 1,2-Dioleoyl-sn-glycero-3-phosphatidyl-L-serine (DOPS);
- (d) 1,2-Dilinoleoyl-sn-glycero-3-phosphatidyl-L-serine (DLPS).

PS analogs were made by phospholipase-D catalyzed exchange of bound choline in respective phosphatidylcholine with serine¹; PS analogs were converted to their calcium phosphate complex² and coated (100µg) on Ti discs (10mm diam., 2mm thick). Human Embryonic Palatal Mesenchymes (HEPMs; the osteoblast progenitors; 1.5 x 10⁴ cells/cm²) were cultured on the coated and uncoated (control) Ti discs for 14 days. Total Protein Production (TPP) and alkaline phosphatase (ALP) specific activity assayed on day-0, 7, & 14.

Biomineralized nodule formation by FRC cells³ was confirmed on Ti coated with best PS analog, by a culture of 30,000 FRCs/disc/well. Time-0 was defined as Day-7 after FRC plating (confluence). On Day-21 after Time-0, samples were washed with PBS and fixed. Nodule formation was evaluated by procion fluorescence, using computer-assisted image analyzer.

Following structural features, relative to their initial osteogenic activities, were studied: 1. the effect of length of fatty acyl (16-C vs 18-C; DPPS vs DSPS); 2. the effect of unsaturation (double bonds) on a constant length fatty acyl at positions-1 & -2 of PS (DSPS vs DOPS vs DLPS). Change in fatty acyl length did not dramatically influence TPP & ALP; inclusion of unsaturation(s) definitely affected both activities significantly. By Day-14, DOPS & DLPS coatings respectively induced almost 226% & 221% as much TPP as recorded for control. Coating of DLPS, with maximum amount of unsaturation on fatty acyl, induced the best expression of ALP. Surface coated with DLPS induced almost 210% as much ALP expression as the control in 14-days. FRCs registered almost 400% as much nodule formation in a 21-day culture on a surface coated with the complex of DLPS, as compared to uncoated Ti.

References:

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