TITANIUM ALLOY COATED WITH PHLOROGLUCINOL-ENRICHED COLLAGEN FIBRILS REGULATES OSTEOGENIC DIFFERENTIATION AND INFLAMMATION

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Introduction

Osseointegration of titanium alloy (Ti6Al4V) is dependent on the promotion of osteoblastic differentiation and reduction of inflammatory response. Collagen coatings can promote adhesion, proliferation and differentiation of osteoblast-like cells [1] and can act as matrices for biological molecules, as polyphenols [2]. Phloroglucinol (PG), the building block of phlorotannins, marine-derived polyphenols, can reduce inflammation and oxidative stress which may hinder osteogenic differentiation [3,4]. Previous studies showed it may have antibacterial properties [5]. This work study the influence of PG-enriched collagen fibrillar coatings on Ti6Al4V on the inflammatory response of fibroblast-like cells and osteogenic differentiation of osteoblast-like cells.

Materials and Methods

Collagen hydrogels were prepared, as described previously [6]. Hydrogels containing PG were also prepared with PG solution of 0.333 mg/mL and 1.0 mg/mL. Ti6Al4V discs, produced as described previously [7], were coated by placing hydrogels on their surfaces, allowing fibrils' absorption. Coatings of collagen and collagen with the lower and higher PG concentrations were named: Ti_Col, Ti_Col_low and Ti_Col_high, respectively, and analyzed by Scanning Electron Microscopy (SEM), X-ray Photoelectron Spectroscopy (XPS) and contact angle (CA) measurements. *In vitro* studies were performed with human osteosarcoma SaOS-2 and mouse embryonic fibroblast 3T3 cell lines and 5×10^4 cells/well were cultured for 3 days on each sample. The gene expression of inflammatory and osteogenic differentiation markers was calculated by real-time polymerase chain reaction.

Results and Discussion

SEM images (FIG. 1) illustrated the presence of collagen fibrils after washing which was confirmed by XPS and CA measurements (data not shown). *In vitro* studies showed that collagen-coated Ti with PG, seem to promote osteogenic differentiation, by an increase of *COL1A1* and *BGLAP* genes with a high PG concentration (FIG. 2a) which are markers of bone matrix production and mineralization regulation, respectively. The expression of *RANKL*, a marker of osteoclast activation and bone resorption, decreased with the coating but when PG concentration increased it increased as well (FIG. 2b). Regarding inflammatory markers (FIG. 3), the coatings reduced their expression. SEM images showed that osteoblast- and fibroblast-like cells attached and spread well on uncoated and coated Ti6Al4V (data not shown).

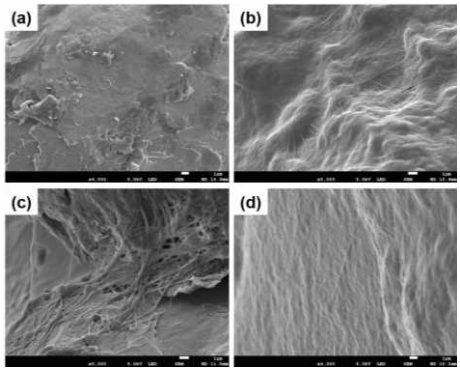

FIG. 1. SEM images (a) uncoated Ti, and (b) Ti_Col, (c) Ti_Col_low and (d) Ti_Col_high coatings.

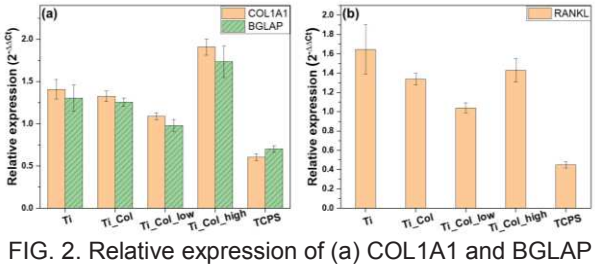

FIG. 2. Relative expression of (a) COL1A1 and BGLAP genes and (b) RANKL gene in osteoblast-like cells.

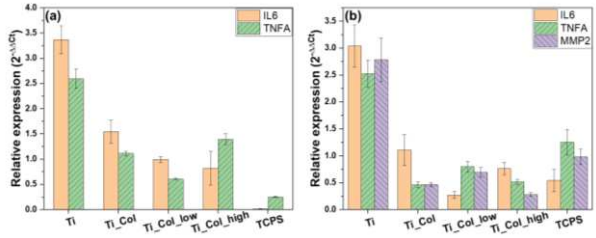

FIG. 3. Relative expression of inflammatory markers in (a) osteoblast-like cells and (b) fibroblast-like cells.

Conclusions

SEM, XPS and CA measurements confirmed the presence of the collagen coatings. *In vitro* tests revealed that PG-enriched coatings may reduce inflammation, promote osteogenic differentiation, and reduce osteoclast activation. Thus, PG-enriched collagen fibril coatings on bone implants are a promising approach.

Acknowledgments

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References

[1] U. Geißler, et al., J. Biomed. Mater. Res., 2000;51;4; 752–760.
[2] T.E.L. Douglas, et al., Biomacromolecules, 2007;8; 1085–1092.
[3] X.C. Bai, et al., Biochem. Biophys. Res. Commun., 2004;314;197–207.
[4] M. Yeo, et al., J. Mater. Chem., 2012;22;3568–3577.
[5] T.E.L. Douglas, et al., Biomed. Mater., 2016;11.
[6] M. Vandrovcova, et al., J. Biomed. Mater. Res. - Part A, 2015;103;525–533.