

HMSC ACTIVITY ON NANOSCALE HYDROXYAPATITE / POLY(LACTIC ACID) COMPOSITE SURFACES

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Introduction

Nanoscale hydroxyapatite (HAp) composite materials have the capacity to enhance mechanical and biological properties of polymeric scaffolds for tissue engineering [Palmer, 2008]. HAp crystal synthesis can be manipulated to alter their morphology, size and crystallinity [Bouyer, 2000]. This work investigates the influence of nano-HAp on 2D composites mechanical properties and human MSC (hMSC) behavior.

Methods

HAp was produced using a sol-gel method [Liu, 2009], in brief Ca^{2+} was titrated into a PO_4^{3-} solution at room temperature and pH was maintained at 10 throughout using a titration device. PLA was dissolved in dioxane and mixed with HAp to a final concentration of 5% polymer v/v and 5%/10% HAp v/v. Composite surfaces were coated on to glass coverslips using a bench top rotary spin coater. HAp crystals were assessed for crystallinity using XRD, composition using EDX and morphology with TEM. Surface roughness was assessed using a stylus profilometer and imaged using SEM. hMSCs were acquired from Lonza Group Ltd. Composite surfaces were seeded 50,000 cells/cm² and alkaline phosphatase (ALP) was measured using a colourimetric assay.

Results

XRD analysis confirmed formation of an amorphous crystallite with associated crystal peaks corresponding to hydroxyapatite (JCPD ref 009-432). EDX evaluation of obtained crystallite confirmed a Ca/P ratio of >1.67. TEM of HAp (Figure 1a) showed predominantly aggregated nanoscale particulates with a plate-like/rod morphology. Profilometry revealed a significant difference in surface roughness between PLA (0.072 ± 0.041µm) and composite surfaces (10% - 2.03 ± 1.00µm & 5% - 1.48 ± 0.786µm). SEM of the composite surface (Figure 1b) shows aggregates of HAp covered by a film of porous PLA. The hMSC's appear to have extended pseudopodia interacting with PLA in lieu of HAp or glass exposed through the pores.

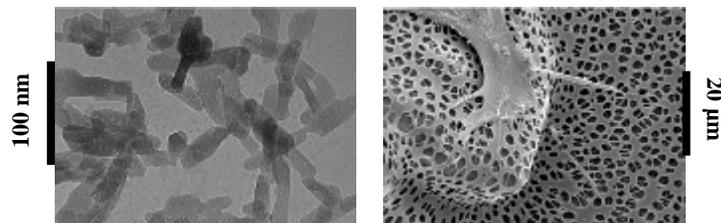


Figure 1: a) TEM of HAp crystals b) SEM of hMSC cultured on composite surface for 24 hours.

hMSC ALP activity appears to be consistent throughout all basal media treatment groups. There was no statistical variation in ALP activity in composite compositions.

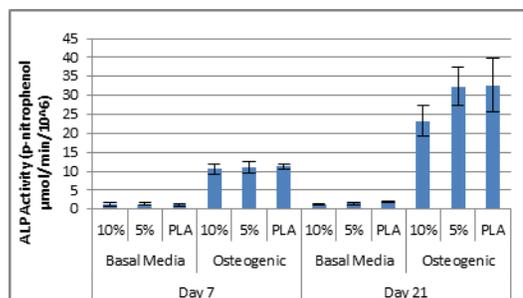


Figure 3: Alkaline phosphatase activity of hMSC cultured on various surfaces for 21 days

Discussion

The composite surfaces contained agglomerated HAp particulates, this could potentially be caused by differences in medium viscosity and Van der Waals forces between HAp crystals. Pores in the polymer surface could be due to humidity during the spin casting process. Differences in ALP activity of hMSC could be due to the surface topography and/or surface stiffness as described by [Discher, 2005].

References

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