Osteoblast Response to Protein Adsorption to 3D Polymer Scaffolds Through Pre-Ageing in Cell Medium

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Statement of Purpose: Protein adsorption mediates biological responses to materials. When a biodegradable polymer scaffold is placed in aqueous, physiological medium, proteins adsorb onto the scaffold surface. The total amount, composition and structure of the adsorbed protein layer can change over time and could influence cell adhesion and proliferation. Thus, we have tested the hypothesis that cell response to a polymeric scaffold is affected by the amount of time that the polymer has been incubated in physiological, serum-containing medium and the amount of protein adsorbed to the scaffold. Previous work has shown that pre-ageing of polymer films in serum-containing cell culture medium enhanced osteoblast adhesion and proliferation [1]. Towards tissue engineering of bone, we examined the effect of "pre-aging time of three-dimensional (3D) polymer scaffolds in serum-containing medium" on 1) amount of protein adsorbed and 2) osteoblast attachment and proliferation.

Methods: 3D poly(ε-caprolactone) (PCL) scaffolds were prepared in 96-well plates by salt-leaching method [2]. PCL solution (relative molecular mass 65000 g/mol, Sigma, 30 µL of 10 % mass/volume in dioxane) was deposited on 0.13 g of NaCl crystals (225 µm- 425 µm diameter) in a 96-well, frozen in liquid N_2, lyophilized and soaked in water to leach the salt. MC3T3-E1 cells (Riken) cultured in α-modification of Eagle's minimum essential medium (Invitrogen) supplemented with 10 volume % of fetal bovine serum (Gibco) and 0.6 % volume % kanamycin sulfate (Sigma-Aldrich) were used as the osteoblast model. To prepare for cell seeding, scaffolds were wetted by adding 0.2 mL medium to each well and centrifuging. Scaffolds were pre-aged for 0 d, 1 d or 7 d at 37 °C to facilitate protein adsorption before replacing with fresh medium containing 10^4 cells. DNA content in the scaffolds was measured by Picogreen (Molecular probes) at 1 d (cell attachment) and 14 d (cell proliferation) culture. Cell nuclei were stained with Sytox Green (Molecular Probes) and imaged. The amount of protein adsorbed to scaffolds after pre-ageing (before cell seeding) was quantified by repeatedly washing the scaffolds with buffer followed by 1 hour incubation with 1 % SDS (sodium dodecyl sulfate). SDS-eluted protein was quantified by BCA assay (bicinchoninic acid).

Results: The total amount of protein adsorbed to scaffolds increased with pre-ageing time (Fig. 1). DNA content (measure of cell numbers, Fig. 2) indicated that cell attachment (1 d) was similar for all pre-ageing times. There was a significant increase in DNA quantity in all scaffolds at 14 d (proliferation). Furthermore, significant increases in DNA content were observed with increase in pre-ageing time. Fluorescence microscopy of nuclear staining corroborated the DNA results.

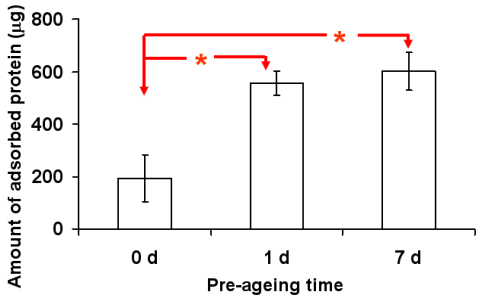

Figure 1: Amount of total adsorbed protein to 3D scaffolds with increased pre-ageing time in serum-containing medium. Statistically significant differences (ANOVA with Tukey's, $p < 0.05$) are indicated by *.

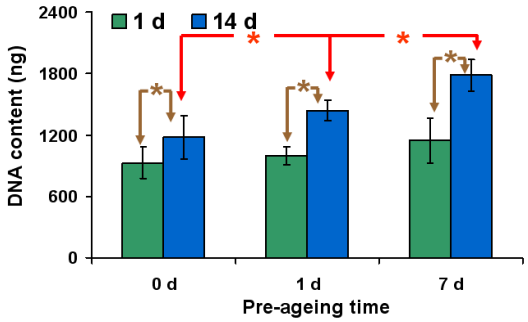

Figure 2: DNA content at 1 d (green) and 14 d (blue) after seeding cells in scaffolds that were pre-aged in serum-containing culture medium. Statistically significant differences (ANOVA with Tukey's, $p < 0.05$) are indicated by *.

Conclusions: The total amount of protein adsorbed to 3D PCL scaffolds increased with increasing pre-ageing time. Increasing pre-aging time did not affect cell attachment to scaffolds at 1 d. However, cell proliferation (14 d) was significantly enhanced with increase in pre-ageing time. These results demonstrate that cell proliferation on polymer scaffolds can be dramatically influenced by pre-ageing time and that pre-ageing time affects the amount of protein adsorbed to scaffolds. Work is currently underway to characterize the total and relative amounts of the serum proteins adsorbed as a result of pre-ageing.

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