Collagen and Mineral Deposition by MLO-A5 Late-Stage Osteoblasts, is Guided by the Fibre Alignment of Electrospun Scaffolds

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Introduction

Mature bone has a highly organised lamella microstructure in which the mineralised collagen fibrils are aligned mostly parallel within a single lamellae but adjacent lamella have orientation at varying degrees from each other. For some osteons this has been described as a 'twisted plywood' structure in which each lamellar has a small difference in angle of orientation from the previous such that the preferred orientation rotates through the layers.

When bone cells are grown *in vitro* on planar substrates there is very little physical guidance of bone structure and hence the matrix is disorganised and probable more representative of the fast-growing immature woven bone. The mechanisms for the collagen fibril alignment is still unclear but some intriguing information comes from tendon research in which cell's primary cilia are aligned along the collagen fibrils of the tendon. In this study we grew late stage osteoblasts MLO-A5s, chosen for their ability to rapidly mineralise in culture and deposit mineral in more physiological structure on fibrous scaffolds that had non-aligned or highly aligned fibre orientations. The aim of the study was to determine whether the fibrous structure would guide cell, primary cilia and subsequent matrix orientation.

Methods

Polymer fibrous scaffolds were synthesised by electrospinning 15wt% medical grade polymer solutions of polycaprolactone (PCL), polyurethane (PU) and PU composites with nano-hydroxyapatite particles (nHA). MLO-A5 cells kindly donated by Lynda Bonewald were seeded onto the scaffolds and their attachment observed by DAPI nuclear stain and phalloidin-TRITC actin stain using confocal microscopy. Primary cilia were stained with anti-alpha tubulin. Collagen fibril orientation was observed over long-term culture and through the depth of the construct using multi-photon excitation and second harmonic generation microscopy. Collagen deposition and mineral deposition were measured by Sirius red and Alizarin red staining respectively and quantified by calorimetry.

Results & Discussion

MLO-A5 cells were shown to align along the polymer fibres in both PCL, PU and nHA composite scaffolds as previously demonstrated for fibroblasts[2]. No alignment was observed in not aligned scaffolds. Cells synthesised collagen aligned in the same direction as the polymer fibres in the first layer of matrix formation and into the depth of the scaffold as observed in PCL scaffolds. Interestingly on the PU and especially the nHA scaffolds, multiple layers of matrix were deposited on top of each other, and in each layer the fibre were parallel to each other but offset by a small angle of about 20˚ in comparison to the previous layer until a height of ~70µm was reached. PU composite scaffolds with nHA produced a higher SHG intensity across all time points indicating a higher amount of collagen deposition on composite scaffolds than on PU-only scaffolds. Primary cilia were orientated at predominantly 40˚ to the PCL fibres. Total mineral deposition was higher by day 21 in constructs with aligned fibres. In conclusion growing osteoblasts on aligned fibres may provide insights into how bone's nano and microstructure is controlled and be a way to create stronger architectures for bone tissue engineering. The primary cilia may have a role in detecting to controlling cell and matrix alignment.

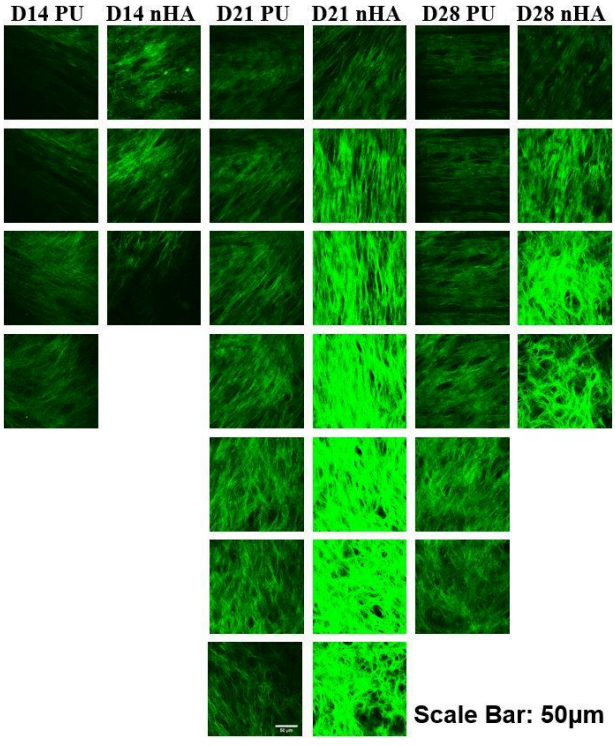

Figure 1: SHG images of collagen deposited by MLO-A5 cells on electrospun polyurethane-only (PU) and polyurethane with nano-hydroxyapatite (nHA) scaffolds; depicting a change in collagen fiber orientation at intervals of ~10µm between images

References

1. Giraud-Guille, M.M. *Calcified tissue int., 42*(3), 167-180. 1988
2. Zhong, S.,et al." *Journal of Biomedical Materials Research Part A, 79*(3), 456-463, 2006

Acknowledgements

The authors would like to thank the Ghana Education Trust Fund for providing financial support to this project.