Effect of protein adsorption on human osteoblast response to porous ferritic fibre networks

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Statement of Purpose: The functionality of implanted devices is dependent on the foreign body reaction that occurs in the tissue surrounding the implant. The initial foreign body response involves the deposition of proteins, primarily fibrin, onto the biomaterial surface. Subsequent cellular responses to a biomaterial surface depend upon this adsorbed protein layer, which in turn depends upon different properties of the material surface, such as chemistry, topography and roughness. The current work investigates the effects of proteins, such as fibrin, on cellular interactions of human bone cells with porous networks of ferritic stainless steel. A type of ferritic stainless steel has been identified that supports the growth, proliferation and differentiation of human osteoblasts[1] without significant inflammatory or cytotoxic responses[2]. This material is intended for use in porous magneto-active layers, made of ferritic fibres bonded together, on the surface of prosthetic implants, which would deform elastically on application of a magnetic field, generating internal stresses within the in-growing bone[3]. Cell responses to the protein-coated stainless steel fibre networks were studied in terms of their attachment, peneration, proliferation and metabolic activity.

Methods: Fibre networks (Bekaert S.A., Belgium) were made from 444 ferritic stainless steel fibres, manufactured by shaving 60 μm fibres off a 100 μm thick sheet. The fibres occupy about 15% of the volume, have a random planar orientation and are bonded together at cross-over points by solid-state sintering. The networks have a Brunauer-Emmett-Teller (BET) surface area of 0.15 ± 0.01 m^2/g.

Human osteoblasts (5.0×10^4 cells per fibre network disc) were seeded in droplets of culture medium supplemented with 0-5 mg/mL fibrinogen (maintained at 37ºC) and subsequently mixed with 0.1 U of thrombin per mg of fibrinogen. Samples were incubated under culture conditions (37ºC, 5% CO_2 atmosphere) until assessment.

Scanning electron microscopy (SEM) was used to evaluate morphology and distribution of the fibrin and cells within the networks. Fluorescence microscopy was further used to image the attachment and penetration of the cells into the protein-coated fibre networks. Cell responses were also assessed through *in vitro* assays. Cellular proliferation and early osteoblastic differentiation were examined using the CyQuant® assay and an alkaline phosphatase (ALP) activity assays, respectively. The alamarblue® assay was used to assess metabolic activity of the cells.

Results: The results of the SEM and fluorescence imaging showed fibrin attachment throughout the networks, bridging between fibres. Cells were embedded in the fibrin, which was composed of nano-scale fibers as shown in Figure 1a. At higher concentrations, sheets of fibrin were produced as shown in Fig 1b.

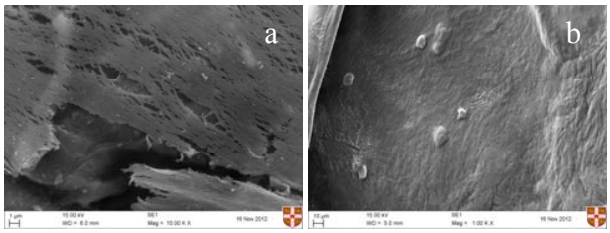

Figure 1. SEM images of fibrin coatings on fibre networks. Fibrin coatings were (a) composed of nanoscale fibres and (b) formed thick sheets with embedded cells.

Fluorescent images further confirmed the cell seeding within the fibrin networks. Cell nuclei were observed between the stainless steel fibres as shown in Figure 2.

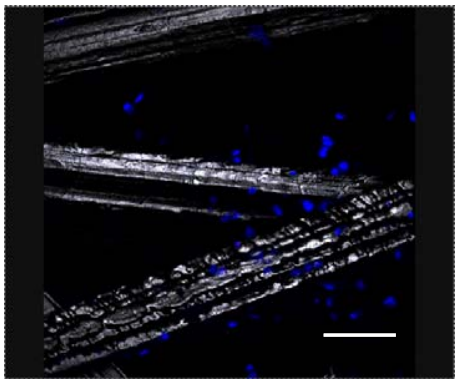

Figure 2. Fluorescent image of cell nuclei stained with DAPI (blue). Stainless steel fibre networks were imaged by reflectance. Scale bar represents 100 μm.

CyQuant and Alamarblue confirmed respectively that the cells had improved proliferation and increased metabolic activity throughout the study on the fibrin-coated samples. Increased activity of alkaline phosphatase indicated the early-stage differentiation of the human osteoblasts within the fibrin-coated fibre networks.

Conclusions: Fibrin adsorption improves cell seeding and proliferation in part by increasing the available surface area for cell attachment within the networks. Fibrin coatings further enhance the cell response of human osteoblasts to these materials. Overall, protein coatings may be useful in developing the potential of ferritic fibre networks for advanced bioengineering using magneto-mechanical stimulation.

References: [1]Malheiro VN, Spear RL, Brooks RA and Markaki AE. Biomaterials. 2011; 32: 6883-6892. [2]Spear RL, Brooks RA and Markaki AE. Journal of Biomedical Materials Research: Part A. 2012: DOI: 10.1002/jbm.a.34451. [3]Markaki AE and Clyne TW. Biomaterials. 2004; 25: 4805-4815.

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