CARBON NANOTUBE-REINFORCED HYDROGELS FOR BONE TISSUE REGENERATION

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[ENGINEERING OF BIOMATERIALS 148 (2018) 61]

Introduction
Whey protein (WPI), a protein derived from dairy sources, and a by-product when producing foods such as Greek yoghurt, has attracted attention for potential applications in tissue engineering. The primary component of WPI is β-lactoglobulin, which is known to promote cell proliferation. Additionally, a recent study [1] demonstrated that cells can adhere, differentiate, and proliferate on WPI hydrogels – which can be fabricated and sterilised in a singular step, by autoclaving. The ability to incorporate particles into hydrogels provides them with further potential advantages for tissue regeneration, including Mechanical reinforcement, antibacterial properties, and further promotion of bone-forming cell proliferation. The inclusion of carbon nanotubes (CNTs) into WPI hydrogels may provide such advantages, as the unique structural, mechanical, electrical and chemical properties of CNTs are already well documented. In this study, CNTs were incorporated into WPI hydrogels, and both the physiochemical and biological properties were evaluated.

Materials and Methods
40% WPI solution (in ddH_2O), was mixed with 0 to 40% CNTs by alternate sonication and vortexing, in Eppendorf tubes. Hydrogels were then simultaneously fabricated and sterilised by autoclaving.
The physical properties of hydrogels were examined using SEM, FT-IR and mechanical testing, while antibacterial tests on *S.aureus* and cell culture was also performed. MG63 osteoblast -like cells were seeded onto hydrogels, before the morphology, proliferation and adhesion of cells were observed at intervals of 1, 4 and 7 days using fluorescent microscopy, coupled with the MTS assay.
All statistical significance was evaluated using one-way ANOVA on SPSS.

Results and Discussion
SEM (Fig. 1) illustrated the successful incorporation of CNTs into the WPI hydrogels. This interaction of WPI with CNTs was further supported by FT-IR (data not shown). Mechanical testing also displayed that the incorporation of CNTs coincided with an elevated Young's modulus, but a decrease in compressive strength (data not shown). Antibacterial testing also displayed a reduction in bacterial growth when CNTs are present. Importantly, even 20% WPI-CNT samples displayed MG-63 cell growth, even after 7 days. Therefore, WPI-CNT hydrogels show promise as a material for future research into bone tissue engineering.

Conclusions
WPI-CNT hydrogels exhibited antibacterial activity against S.aureus, and growth of MG63 cells was not apparently worse on WPI-CNT hydrogels. Finally, the addition of CNTs resulted in stiffer hydrogels with lower compressive strength.

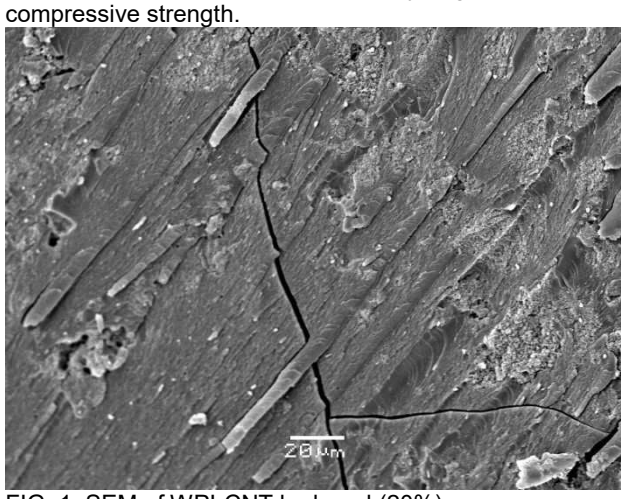

FIG. 1. SEM of WPI-CNT hydrogel (20%).

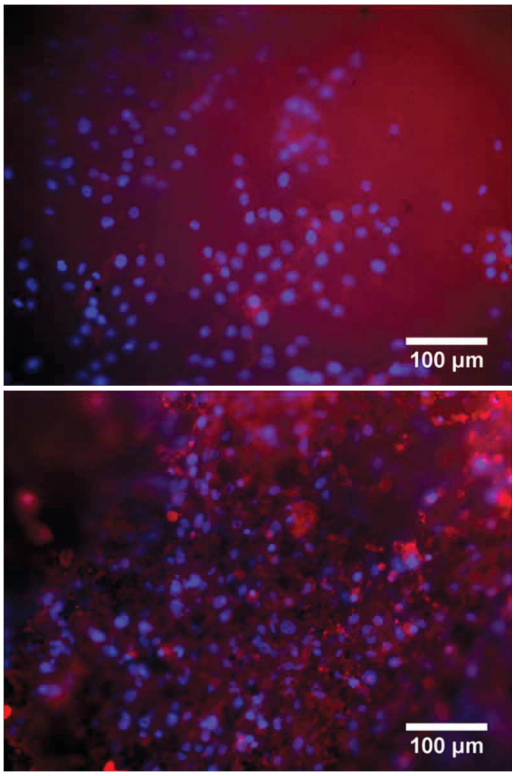

FIG. 2. Fluorescent microscopy of WPI control (left) and WPI-20%CNT (right) after cell culture with MG-63 cells (images after 7 days, red- Texas red, Blue- Hoescht stain).

Acknowledgments
United Kingdom Society for Biomaterials Lab-2-Lab grant N8 Agrifood pump priming grant "Food2Bone" (T.E.L.D., J.H., R, D'S.)),

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