OSTEOBLAST BEHAVIOUR ON NOVEL WHEY PROTEIN ISOLATE HYDROGELS

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Introduction

The first critical course for assessing the suitability of a new material in bone regeneration is the compatibility of the biosystem at the site of its effect. Knowledge of the molecular interactions material modifications with the biosystem is of specific importance.

Proteins derived from dairy sources have attracted attention for regenerative medicine. Whey protein isolate (WPI) is a by-product from the manufacture of Greek yoghurt. It contains mainly of β-lactoglobulin. Hydrogels are highly hydrated three-dimensional polymer networks which are used increasingly as materials for bone tissue regeneration. WPI hydrogels are formed by heating WPI solution, a well-known phenomenon in the food industry.

Osteoblasts are the important cell type in orthopedic applications. The most important initial process in this cell-material interaction is the mechanical anchoring of the cell to the material interface – the cellular adhesion (FIG. 1) [1]. External signals from physico-chemical environments finally affect the cell physiology [2]. Therefore, it is important to study *in vitro* effects of hydrogel composition on cellular adhesion, and cell growth, organization of the actin cytoskeleton, and proliferation [2].

Previous work has shown that WPI improves cell proliferation and osteogenic differentiation [3]. The application of WPI hydrogels in tissue engineering has still not been explored. The understanding and interpretation of the cellular behaviour is critical for the acceptance of novel materials on bone regeneration.

Materials and Methods

WPI hydrogels were formed by autoclaving WPI solution, i.e. by performing fabrication and sterilization in one step. Various WPI concentrations (20, 30, 40, 50% all w/v) were compared and subjected to compressive testing. Young's modulus increased with rising WPI concentration (FIG. 2). The first *in vitro* studies, to conduct the acceptance of novel whey protein isolated hydrogels, were performed with osteoblasts (MG-63, ATCC® CRL-1427™). The cells were cultured within 24h in DMEM with 10% FCS (Biochrom) under physiological conditions: 37°C, 5 % CO_2 [2]. To analyse the cell adhesion and growth we used microscopy – scanning electron microscopy (FE-SEM), confocal laser scanning microscopy (LSM), and flow cytometry. To validate the data we used GraphPad Prism with the corresponding test of significance.

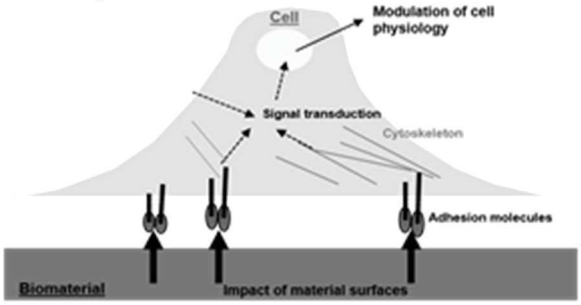

FIG. 1. Scheme of cell-material interaction.

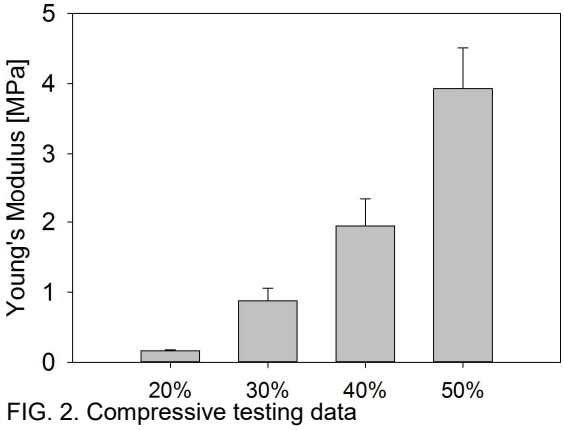

FIG. 2. Compressive testing data

Results and Discussion

Established on our *in vitro* studies, we were able to demonstrate that changing the concentration of whey protein hydrogels influences the osteoblast behavior: changes in cell morphology, the organization of cellular structures, and finally proliferation. Morphological analyses with FE-SEM revealed the cellular behaviour on the hydrogels; this is an important parameter. Using LSM we were able to recognize the influence of hydrogels on the spatial organization of cellular components, e.g. the actin cytoskeleton. Cell growth and spreading occurred on hydrogels, particularly on 40% and 50% hydrogels compared to 20% and 30% hydrogels. These studies of the cell physiology and cell adhesion are first important steps for assessing cellular behavior at the interface of a material with a biological environment.

Conclusions

WPI hydrogels show potential as biomaterials for bone tissue engineering. Further work will focus on osteogenic differentiation studies. Cell biologic *in vitro* studies are necessary for a better understanding and assessment of innovative medical materials and their interplay with the surrounding biosystem. A material for bone tissue engineering should support attachment and proliferation of bone-forming cells.

Acknowledgments

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