Whey protein isolate (WPI): a versatile dairy-derived hydrogel for bone and vascular tissue engineering and antimicrobial applications

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INTRODUCTION: Whey Protein Isolate (WPI) is an inexpensive by-product of the dairy industry, available in large quantities and used as a dietary supplement. WPI is over 97% protein; three-quarters is beta-lactoglobulin (β-LG) [1]. WPI in cell culture medium promoted the proliferation and differentiation of bone-forming cells [2]. Solutions of WPI can be heated to form hydrogels, which withstand sterilization by autoclaving; an important practical advantage. Denaturation of β-LG leads to increased hydrophobic interactions and disulphide bond formation and thus crosslinking to form the polymer hydrogel network [3]. We have studied WPI hydrogels as scaffolds for bone-forming cells and carriers of hydrophobic substances.

EXPERIMENTAL: WPI hydrogels 15% to 40% (w/v) have been made [4-5]; inorganic particles, like bioactive glasses, alpha-tricalcium phosphate, aragonite and hydroxyapatite (HA) can easily be added during hydrogel formation [6-9]. Hydrophobic molecules such as phloroglucinol (PG), the fundamental subunit of marine polyphenols, and poly-gamma-glutamic acid (PGGA), can be incorporated during hydrogel formation [11-12].

RESULTS AND DISCUSSION: WPI hydrogels support the adhesion and growth of a range of bone-forming cells, including MG-63 osteoblast-like cells, normal human foetal osteoblasts (hFOB) normal mouse calvarial preosteoblasts (MC3T3-E1) and dental pulp stem cells [6-10], as well as human umbilical vascular endothelial cells (HUVEC) [5]. Addition of aragonite [8] and PGGA [11] promoted osteoblastic differentiation, while incorporation of PG endowed antimicrobial activity towards a wide range of microbes including methicillin-resistant Staphylococcus aureus and Staphylococcus epidermidis while maintaining cytocompatibility [11].

CONCLUSIONS: WPI hydrogels are both promising scaffolds for bone cells and hydrophobic drug carriers.

REFERENCES

[1] Keppler J. K. et al., Differences in heat stability and ligand binding among β-lactoglobulin genetic variants A, B and C using (1)H NMR and fluorescence quenching *Biochim Biophys Acta* 2014;1844(6):1083-93

[2] Douglas, T.E.L et a., Application of whey protein isolate in bone regeneration: Effects on growth and osteogenic differentiation of bone-forming cells *J Dairy Sci.* 2018; 101(1):28-36

[3] Orlien V., Utilizing High Pressure Processing to Induce Structural Changes in Dairy and Meat Products, *Reference Module in Food Science* 2016, First Edition, 1–9

[4] Norris, K. et al. Marine-inspired enzymatic mineralization of dairy-derived whey protein isolate (WPI) hydrogels for bone tissue regeneration *Marine Drugs* 2020; 18, 6-18.

[5] Genç, H. et al. Endothelialization of Whey Protein Isolate-Based Scaffolds for Tissue Regeneration, *Molecules* 2023, 28(20), 7052

[6] Dziadek, M. et al. Modification of heat-induced whey protein isolate hydrogel with highly bioactive glass particles results in promising biomaterial for bone tissue engineering, *Mater Des* 2021; 205, 109749

[7] Dziadek, M. et al. Novel multicomponent organic-inorganic WPI/gelatin/CaP hydrogel composites for bone tissue engineering, *J Biomed Mater Res A* 2019; 107, 2479-2491.

[8] Gupta, D. et al. Novel naturally derived whey protein isolate and aragonite biocomposite hydrogels have potential for bone regeneration, *Mater Des* 2020; 188, 108408

[9] Klimek K. et al. Could Curdlan/Whey Protein Isolate/Hydroxyapatite Biomaterials Be Considered as Promising Bone Scaffolds?—Fabrication, Characterization, and Evaluation of Cytocompatibility towards Osteoblast Cells In Vitro, *Cells* 2022; 11(20), 3251

[10] Platania V. et al. Phloroglucinol-enhanced whey protein isolate hydrogels with antimicrobial activity for tissue engineering, *Mater Sci Eng C* 2021; 129, 112412

[11] Baines, D., et al. The enrichment of whey protein isolate hydrogels with Poly-γ-glutamic acid promotes the proliferation and osteogenic differentiation of pre-osteoblasts, *Gels*, accepted 19/12/2023

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