In Vitro Evaluation of the Osteoinductive Potential of Chitosan Membranes Loaded with Raspberry Ketone

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INTRODUCTION

Guided bone regeneration (GBR) membranes are used to guide healing in oral bone defects by preventing soft tissue invasion of regenerating bone sites. Electrospun chitosan membranes (ESCM) have exhibited potential for use in GBR applications[1]. ESCM also have potential for local delivery of therapeutics for enhancing healing due to high surface area of the nano-fibers1. Raspberry ketone (RK) is a simple compound found in berries that has exhibited osteogenic potential in vitro[2]. RK can be extracted from berries, making it inexpensive to manufacture compared to bone morphogenetic protein 2 (BMP-2), which is currently used to stimulate bone growth. This work aimed to evaluate effect of RK released from ESCM on growth, osteodifferentiation, and calcium-phosphate deposition of a mouse marrow cell line. Experiments were conducted using osteogenic medium with and without BMP-2 to evaluate interactions between released RK and BMP-2 that would be present naturally in healing defects.

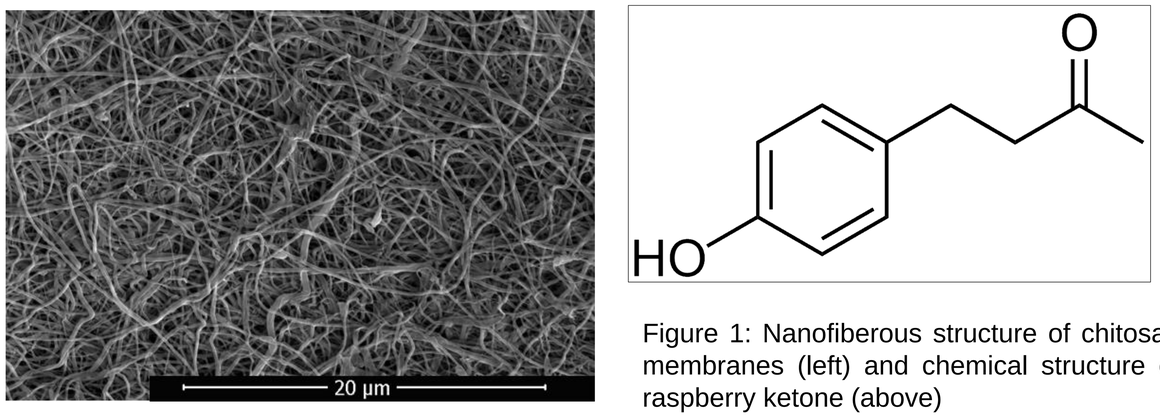

Figure 1: Nanofiberous structure of chitosan membranes (left) and chemical structure of raspberry ketone (above)

METHODS

W-20-17 cells (ATCC-CRL2623), mouse stromal cell line, were used. Chitosan (70% DDA Primex, Iceland) was elecrospun, cut into discs 1cm in diameter, and modified with hexanoic anhydride to provide 10-12 days of RK release as previously reported[3]. Cells were seeded into 24 well plates at 27,000 cells/cm^2 and cultured in DMEM with 10% FBS, 1% penicillin, streptomycin, and neomycin (Thermo Fischer, MA), 50µg/ml ascorbic acid, 5mM β-glycerophosphate, and 25ng/ml BMP-2. RK was added to ESCMs at 0, 100, 250, or 500 µg RK/ESCM and placed in cell culture inserts. Negative controls contained no RK or BMP-2 (TCP control) and positive controls contained 25ng/ml BMP-2 in medium and ESCM with 0µg RK. Cells were assayed at 1, 7, 14, and 21 days for growth based on dsDNA (Invitrogen Quant-iT dsDNA Assay Kit, CA), ALP (BioAssay Systems QuantiChrom ALP Assay Kit, CA) and calcium phosphate deposition (Calcium Reagent Kit, Pointe Scientific, MI) at day 14 and 21. ALP was normalized to dsDNA. ANOVA and post hoc tests were used for statistical analyses (α=0.05; n=4/grp/time point).

Acknowledgments
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References
[1] Murali VP. Int J Pharm. 2020;584 119438
[2] Takata T. J Med Food. 2014;17(3): 332-338
[3] Cameron PK. MS Thesis. University of Memphis; 2019.

RESULTS

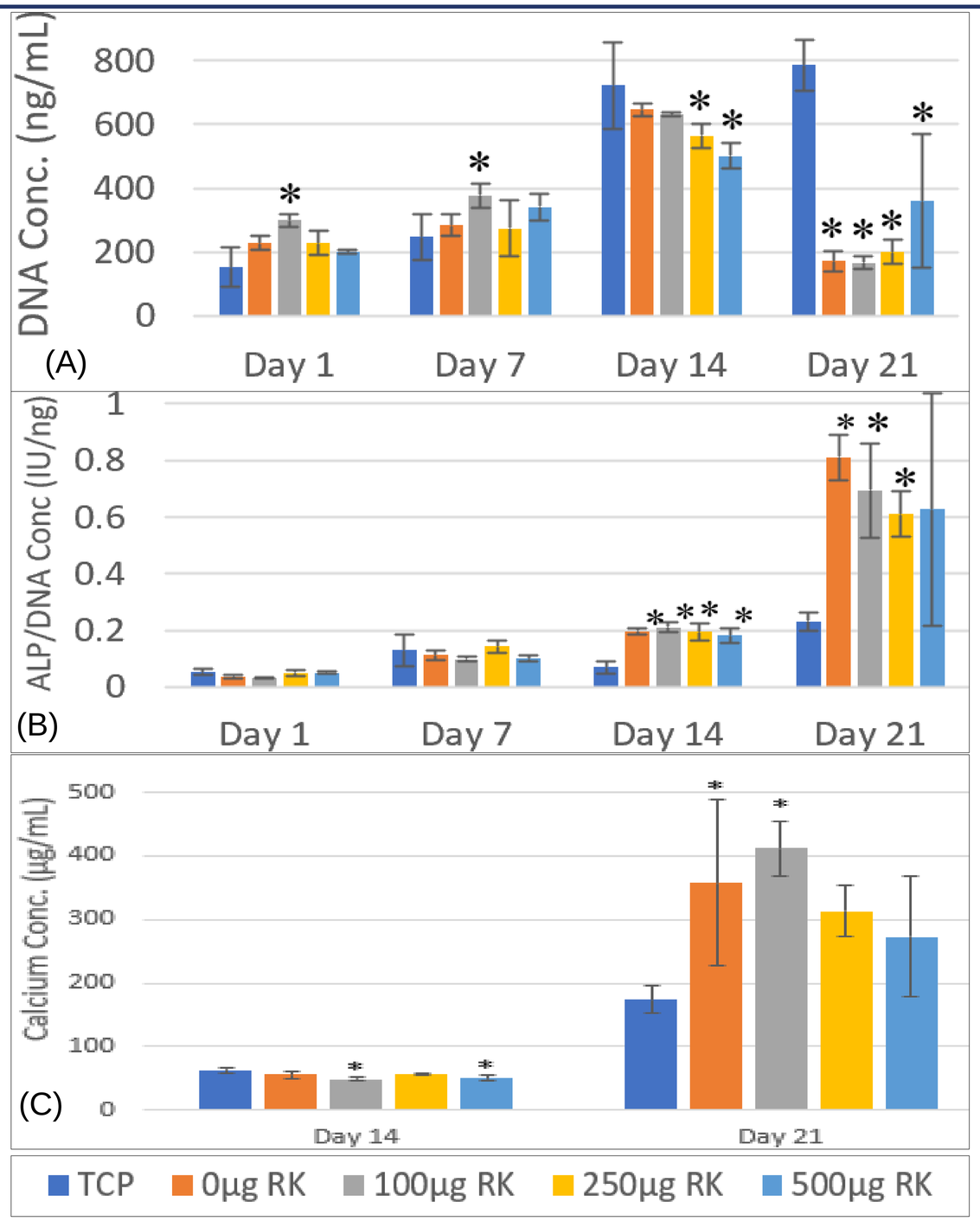

Figure 2: Cellular DNA content (A), alkaline phosphatase production normalized to DNA (B), and calcium concentration (C) of W-20-17 cells exposed to BMP-2 and/or raspberry ketone

CONCLUSIONS

At day 14 cells in positive control and RK ESCM groups began to differentiate as evidenced by increased ALP compared to negative control. The decreased number of cells at day 21 for positive control and RK ESCM group correspond to increased mineralization. Negative controls did not mineralize and continued to proliferate. However, lack of difference in ALP and Ca deposition between the BMP-2 control and RK ESCM groups may indicate RK had minimal effect on differentiation. However, BMP-2 was maintained at constant dose, whereas RK was quickly eluted and eliminated from culture due to rapid released from ESCMs over the initial 3-5 days, followed by decreasing release until day 10-12[3]. Previous experiments indicated additive effect of BMP-2 and 25-100µg/mL RK on ALP and Ca deposition of W-20-17 cells when both were added to culture medium. Improving and extending release profile of RK from ESCM may lead to improved osteogenic potential.