Confidential to United Fisheries Limited

Summary of findings:

The effect of a United Fisheries Limited shark cartilage powder on osteoblast mineralisation

Dr Wei-Hang Chua

Mrs Gabrielle Plimmer

Prof Marlena C Kruger

Bone Health Research Group
Institute of Food, Nutrition & Human Health
College of Health
Massey University
Private Bag 11 222
Palmerston North 4442
New Zealand

Phone +64 6 350 5799 extension 83326
Email: w.h.chua@massey.ac.nz

February 2015

Confidentiality Classification

This report is classified as:

☐ Unrestricted – freely available

☑ Confidential to United Fisheries Limited

☐ Restricted Distribution

☐ Customer Only

(Sign)

Principal Investigator, Physiology Division, IFNHH, Massey University

Copy 1 of 1

The contents of this publication are confidential to and the property of United Fisheries Limited and, without prior consent, may not be disclosed to a third party or copied or used in any manner not authorised by the aforementioned.

Lay Summary

Aims:

This study was designed to test the effect of a United Fisheries Limited Shark cartilage powder on:

Mineralisation in osteoblast-like cells.

> *What effect does the United Fisheries Limited shark cartilage powder have on mineralisation of osteoblast bone cells? Osteoblasts are the cells which build bone. Does this powder stimulate or inhibit the production of bone mineral nodules by these cells?*

What we did:

We used osteoblast precursor cells grown in the lab. It is accepted practice to use mammalian cells, in this case a mouse cell line. We used a mouse osteoblast cell called MC3T3-E1 subclone 4. This cell line is a well established model used to demonstrate osteoblast functions and activities. We treated these cells with the United Fisheries shark cartilage powder to test the above aim. This is the same process as would be used with the first steps of testing factors or drugs that might have a positive effect on bone growth or a protective effect from bone breakdown.

What we found:

The United Fisheries shark cartilage powder had a significant effect on bone cells in the lab, indicating potential positive effects on bone cell function.

The shark cartilage powder had a significant positive effect on osteoblast mineralisation. This means that the powders caused the bone-making osteoblast cells to produce more bone mineral, although it should be noted that this effect was small. The powder did not inhibit and was therefore not toxic to this bone formation process in this model.

Recommendations:

The main findings of the latest study, along with the results from the previous reports show:

- The United Fisheries shark cartilage powder has a small effect on increasing bone formation via osteoblast differentiation and mineralisation.

Scientific Summary

This study was designed to assess the effect of a United Fisheries shark cartilage powder on osteoblast function. We have previously tested and reported that this powder stimulates osteoblast differentiation, and this study assesses whether differentiation progresses to mineral nodule formation by these cells.

The study used the mouse MC3T3-E1 cell line as an in vitro osteoblast model. This cell line is a well-established models used to demonstrate osteoblast phenotypic activities.

1. Mineral nodule quantity as assessed by Alizarin Red staining was increased in MC3T3-E1 cells after treatment with a United Fisheries shark cartilage powder after 17 and 25 days (Figure 1 and 2).
2. There was a significant effect of the shark cartilage powder on bone nodule formation (as measured by Alizarin Red staining; Figure 2) after 17 days of treatment at a test concentration of 10 µg/ml, and at 0.1 and 100 µg/ml by 25 days of treatment. Each experiment was repeated three times, so further replication could confirm if activity is at only these test concentrations or if the effect is wider.
3. While the powder had a significant effect on bone cells in vitro, the powder was only partially soluble in solution (i.e. there was a proportion of powder which was insoluble).
4. The soluble portion of the powder had some effect on the bone cell function models used indicating bioactivity of some of the soluble components.
5. To further investigate the effects of this powder, it is recommended that the powder solubilisation process is replicated and samples of the soluble fractions are submitted for chemical analysis for (i) protein, (ii) calcium, (iii) magnesium, (iv) phosphorus, (v) collagen, (vi) glycosaminoglycan, (vii) chondroitin sulphate, and (viii) fat. Including respective blank controls for each of the cell culture medias used.
6. Subject the powder to a simulated gastric digest *in vitro*. Using a method compatible with the osteoblast model, this could completely digest the entire powder sample (instead of the soluble part only) in a form similar to what would be presented to the gut.

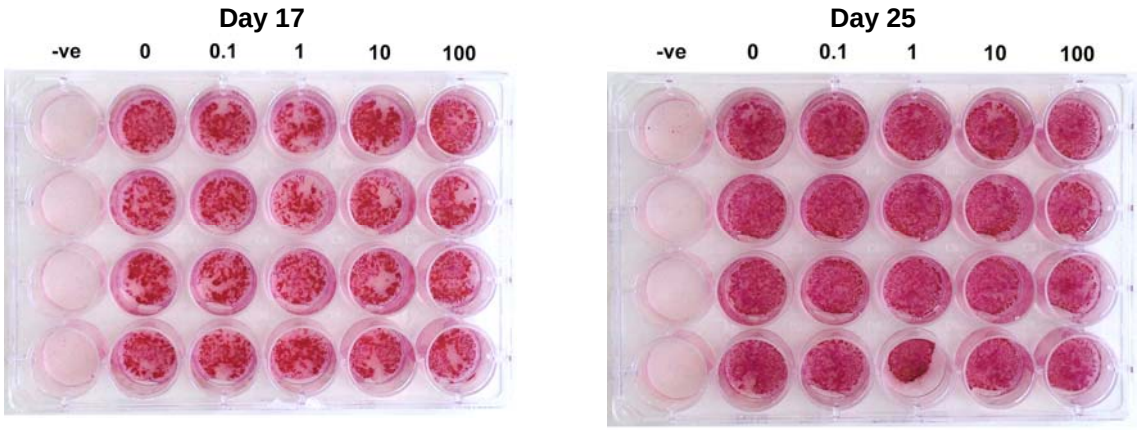

Figure 1. 24-well tissue culture plates showing mineral nodule formation by osteoblasts after 17 and 25 days of treatment with shark cartilage powder. Cells were incubated with solubilised powder samples on a protein concentration basis at 0.1, 1, 10 and 100 µg/ml for 17 and 25 days in the presence of osteogenic cell culture media. Cells were also grown in the absence of osteogenic media to serve as a negative control (-ve). To assess mineralisation, each well was stained with Alizarin Red to stain mineralised nodules (pink/red staining).

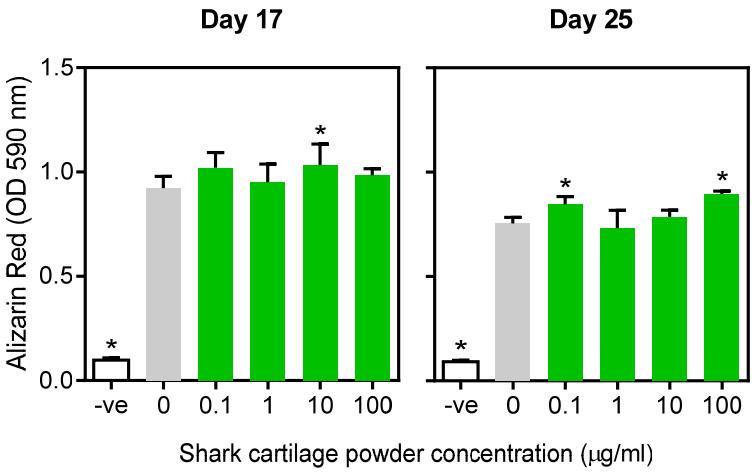

Figure 2. Alizarin red activity in Mc3t3-E1 subclone 4 osteoblast cells treated with shark cartilage powder. Cells were incubated with solubilised powder samples on a protein concentration basis at 0.1, 1, 10 and 100 µg/ml for 17 and 25 days in the presence of osteogenic cell culture media. Cells were also grown in the absence of osteogenic media to serve as a negative control (-ve). Alizarin red activity reflects the degree of mineralisation in each treatment. Results are shown as means ± 95% confidence intervals (CI). An asterisk is used to indicate statistical significant ($p < 0.05$) of the indicated treatment compared to 0.