The effect of a United Fisheries Limited shark cartilage powder on osteoblast function and osteoclast-precursor differentiation

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Bone is a dynamic and living tissue that grows, repairs and degenerates through the activity of two main cells. Bone cells called osteoblasts are important for laying down bone and their activity has been shown to be influenced by dietary factors. Other bone cells called osteoclasts, break down the bone matrix. In the body, osteoblasts and osteoclasts work in concert so that bone can grow (osteoblast activity greater than osteoclast activity), repair, or degenerates (osteoblast activity less than osteoclast activity), the latter occurs in diseased state or in the latter stages of the life cycle. In the human lifecycle good "bone" nutrition can increase a person's maximum possible skeletal bone density, i.e. maximising the "osteoblast activity greater than osteoclast activity". In latter life when "osteoblast activity is less than osteoclast activity", pharmaceutical agents and now increasing nutrition are being looked at as ways to reduce osteoclast activity and therefore bone loss.

Aims

This *in vitro* study was designed to test the effect of a United Fisheries Ltd shark cartilage powder on:

1. Proliferation and differentiation of osteoblast-like cells. *Does the powder cause osteoblasts to grow and multiply (proliferate) and develop characteristics of cells which could produce bone matrix (differentiate) or does the powder prevent this from happening?*
2. Differentiation of osteoclast precursors into osteoclasts. *What effect does this powder have on osteoclast precursor cells, does the powder encourage or inhibit this process?*

What was done

We used osteoblast and osteoclast precursor cells grown in the lab. We used the MC3T3-E1 subclone 4 mouse osteoblast cell line, and a mouse osteoclast precursor cell celled the RAW 264.7 macrophage. Both cells are well established models used to demonstrate osteoblast and osteoclast functions and activities. We treated these cells with the powders to test the above aims. This is a similar 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.

After being treated with the powder, osteoblast cell viability and numbers were measured using the methyl-thiazolyl tetrazolium (MTT) assay and differentiation quantified by measuring alkaline phosphatase. Osteoclast pre-cursors were treated with the powder in the presence of receptor activator of nuclear factor kappa-B ligand (RANKL) which triggers osteoclast formation. To test the effect of the shark cartilage powder on osteoclast formation, multinucleated cell (MNC) formation and tartrate-resistant acid phosphatase levels (an osteoclast marker) were measured. Data was analysed using one-way analysis of variance followed by post-hoc testing.

Results and findings

The effect of Shark Cartilage powder on osteoblast cell proliferation

- Shark Cartilage Powder was not toxic to the osteoblast bone cells at the tested concentrations (Figure 1).
- Shark cartilage powder enhanced the growth of the osteoblast cells, and under this treatment these cells showed indications of maturing into a form which could produce bone mineral (Figure 2).

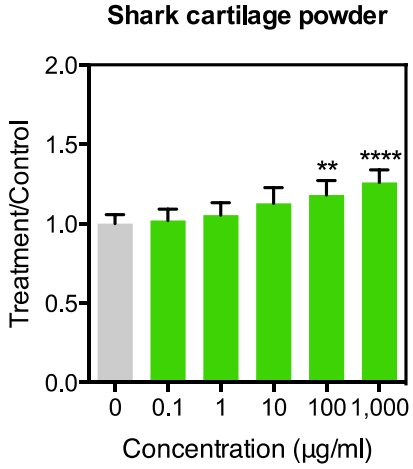

Figure 1. Effect of a shark cartilage powder on Mc3t3-E1 subclone 4 osteoblast cell proliferation. Statistical significance is indicated by * p = 0.01 to 0.05, ** p = 0.001 to 0.01, *** p = 0.0001 to 0.001, and **** p < 0.0001. Results are mean ± 95% CI.

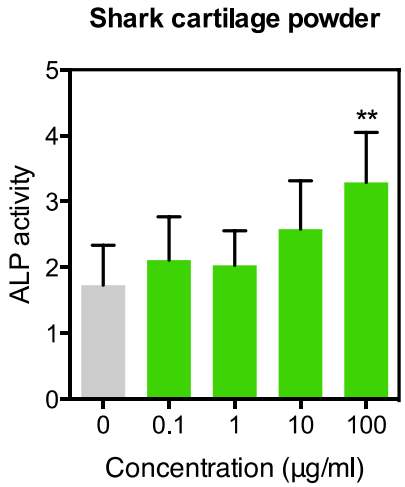

Figure 2. Effect of shark cartilage powder on differentiation of Mc3t3-E1 subclone 4 osteoblasts. Statistical significance is indicated by * p = 0.01 to 0.05, ** p = 0.001 to 0.01, *** p = 0.0001 to 0.001, and **** p < 0.0001. Results are mean ± 95% CI.

The effect of Shark Cartilage powder on osteoclast formation

- Shark Cartilage Powder did not reduce or inhibit the amount of osteoclasts (bone dissolving cells) that formed (Figure 3).

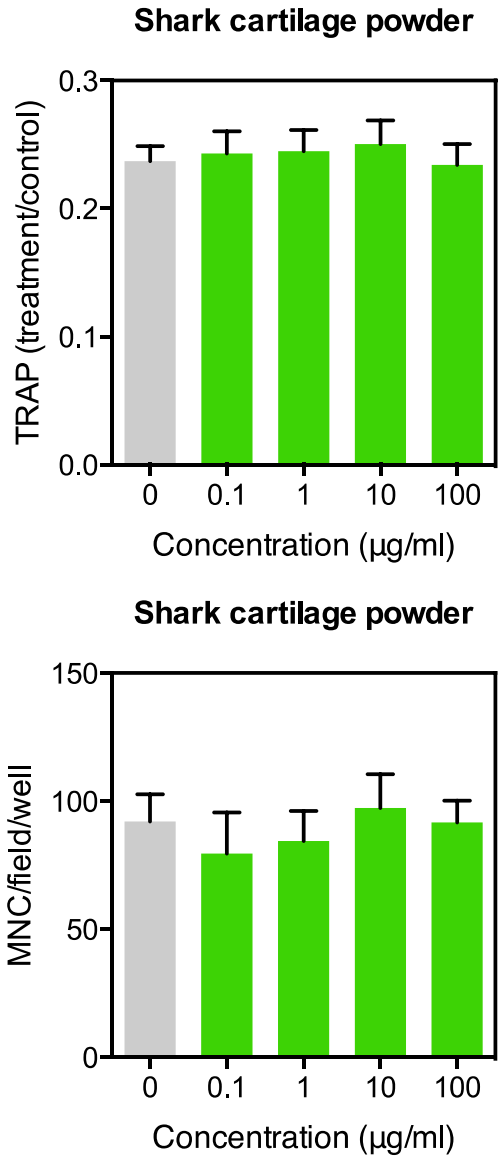

Figure 3. Effect of a shark cartilage powder on tartrate-resistant acid phosphatase (TRAP) activity and average MNC cell number per photomicrograph field per well in RANKL-treated RAW 264.7 cells. Statistical significance is indicated by * p = 0.01 to 0.05, ** p = 0.001 to 0.01, *** p = 0.0001 to 0.001, and **** p < 0.0001. Results are mean ± 95% CI.