2014

The effects of mineral microparticles on dental cell differentiation

A. Farley

K. Shores

Z. Messick

D. Dean

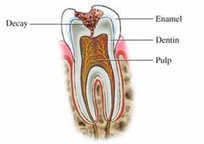
M. Kennedy

The Effect of Mineral Microparticles on Dental Cells

A. Farley[1], K. Shores[1], Z. Messick[1], M. Kennedy[2], L. Datko[1], D. Dean[1]

Department of Bioengineering[1] and Department of Materials Science and Engineering[2], Clemson University

Introduction



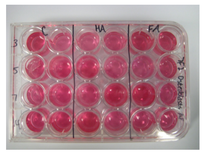
A tooth exhibiting dental caries, a cavity, in the crown. gurgaondentist.blogspot.com

A hockey player missing a tooth http://austincosmeticdentistry.com

- With the growth of regenerative medicine, the use of natural tooth fillers that can stimulate host tissue growth has become more popular.
- Recent research delves into effects of adding apatite minerals to osteogenic cells to stimulate bone growth[2].

Materials and Methods

- Cells studied: bone forming cells (7F2 Osteoblasts), bone marrow stem cells (BMSCs), and dental pulp stem cells (DPSCs).

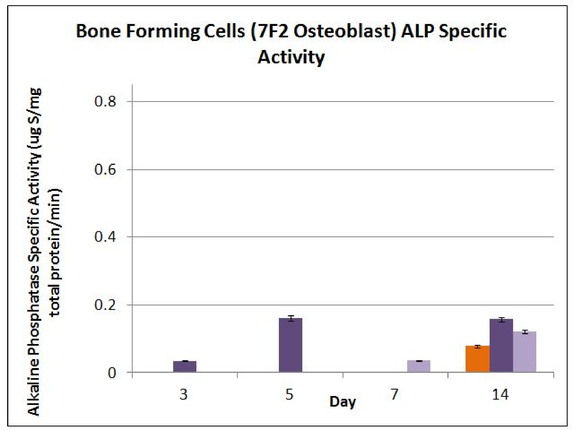


Cells culturing in an 24 well plate.

- Standard growth media: AlphaMEM with 15% FBS and 5% pen/strep.
- Mineral microparticles: Hydroxyapatite (HA) and Fluorapatite (FA).
- Determined total protein content by running bicinchonic acid (BCA) assays.
- Determined ALP specific activity by running alkaline phosphatase assays (ALP). ALP is an early marker of mineral cell differentiation.
- Use Alizarin Red staining to find areas of calcification.
- Used antibody stains to determine the presence of osteocalcin and collagen I.
- Examined cell-particle interactions.

Results

- HA (hydroxyapatite) cells had higher levels of osteocalcin and collagen I in the first five days of culture than did both FA (flurapatite) and control cells.
- DPSCs responded best to being cultured with microparticles (Figure 1). Cells cultured with HA had higher levels of ALP in the first 5 days than did the others, but after 5 days the presence of either type of microparticle hindered ALP specific activity.
- Cells tended to congregate in large clusters around HA particles (Figure 2), but in smaller groups around small clusters of FA particles. It was not uncommon to see one or two cells next to a FA particle.



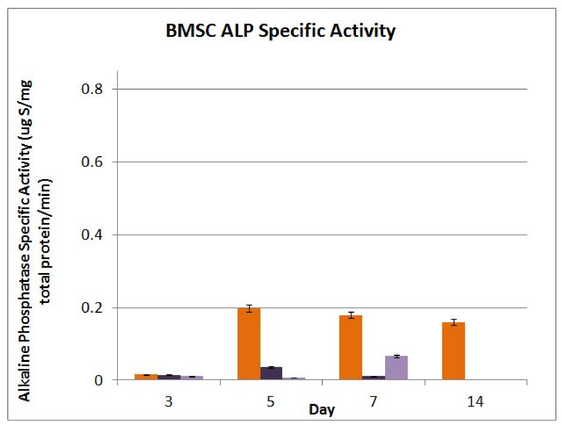


Figure 1: Graphs showing the Alkaline Phosphatase (ALP) specific activity for bone forming cells (top left), bone marrow stem cells (bottom left), and dental pulp stem cells(right).

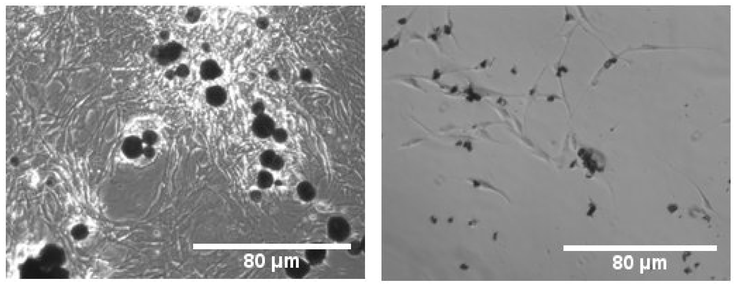
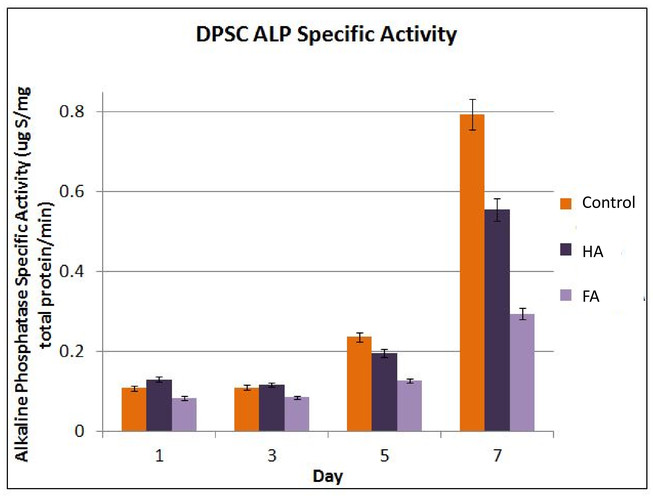


Figure 2: Cell-Particle Interactions of bone forming cells with HA particles (left) and bone forming cells with FA particles (right).



Discussion and Conclusions

- Cells reacted better to being cultured with HA particles than FA particles (Fig. 2).
- The data showed that HA stimulated protein and ALP levels in the first 5 days, then inhibited levels after.
- HA cell cultures showed higher levels of ALP specific activity (Fig .1), osteocalcin, and collagen I than FA.
- Though the antibody stain results were scattered, they still followed the trend of HA cells having higher levels of protein.
- Overall, the results show a lower differentiation potential for cells that were exposed to mineral microparticles. This was an unexpected result and contradicts data shown in the literature[1]. While it is known that the chemical compositions of the partilces have an effect of cell differentiation potential, the effect of the size of the particles is not clear. Out particles were on a much larger scale than in previously performed experiments which may have affected cell differentiation.

Current and Future Work

- Currently performing polymerase chain reaction assays for dentin sailophosphoprotein (DSPP)and secreted phosphoprotein (SPP)recognition to further quantify cell differentiation.

Acknowledgements

- Funding from NIH (Award number: K25HL 902228) and the Clemson University Creative Inquiry program including, Anne-Miller Adams, Katherine Hafner, Brian Kirkland, and Diane Zybko. The authors would also like to thank Matt Rusin, Ruikai Chen, and Sooneon Bae for their technical support.

Selected References

[1] Pines A et al. Clinical trial of microcrystalline hydroxyapatite compound (‘Ossopan’) in the prevention of osteoporosis due to corticosteroid therapy. Curr Med Res Opin 1984.

[2] Webster TJ et al Osteoblast adhesion on nanophase ceramics. Biomaterials 1999.