Increased Expression of Osteogenic Markers and Bone Mineral Formation in Response to Metal-Doped Bioactive Glass

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Introduction: Bioactive glass (including Bioglass trademark) is a potentially useful biomaterial for orthopaedic and dental implant applications. Bioactive glass materials have been shown to stimulate osteoblast activity in vitro (Kaufmann EA. J Biomed Mater Res, Part A. 2000:52A:783-796) and also to have antimicrobial and anti-inflammatory properties in vivo. Osteoblasts are the primary bone-forming cells and are critically important in fracture healing, total joint replacement, and integration of implants with the surrounding bone. Osseointegration requires adhesion between the osteoblasts and the material surface as well as proliferation and differentiation. It has been shown that expression of mature osteoblast markers can be regulated by the presence of metal ions in the tissue culture media (Vrouwenvelder WC. Biomaterials. 1994:15:97-106. and Oki A. J Biomed Mater Res, Part A. 2004:69A:216-221). However, other research indicates that certain transition metal ions are toxic to osteoblasts (McKay GC. Biomaterials. 1996:17:1339-1344). The purpose of this study is to investigate the effects of metal doping on osteoblast viability and activity in response to bioactive glass stimulation.

Methods: Bioactive glass compositions were based on the 45S5 bioactive glass which contains at least 45% SiO_2. A variety of metal-doped bioactive glasses were produced by adding trace amounts of chromium, nickel, vanadium, iron, or copper to the molten glass. Particles were then produced using the sol-gel processing method. MC-3T3 E1 pre-osteoblast cells were maintained using standard culture conditions. Viability testing was performed using a trypan blue dye exclusion test 24 hours after exposure to various doses of bioactive glass. Osteoblast activity was measured by alkaline phosphatase specific activity assay or mineralization activity assay. For both of the activity assays, MC-3T3 cells were cultured in osteoblast differentiation media supplemented with inorganic phosphate and dexamethasone. Bioactive glass was added to the cultures at a dose of 1 mg/mL. Alkaline phosphatase specific activity was measured 4, 7, and 10 days after bioactive glass stimulation. Mineralization activity was assessed by alizarin red staining 7, 10, or 14 days after stimulation.

Results and Discussion: Initial experiments with the bioactive glass showed toxicity when MC-3T3 cells were stimulated with 100 mg/mL bioactive glass (including the undoped controls). This toxicity was dose-dependent and no differences in viability were seen using 1 mg/mL bioactive glass when compared to undoped bioactive glass and control (untreated) cells. Among the different types of bioactive glass used, the chromium-doped bioactive glass exhibited the most cytotoxicity, as expected. MC-3T3 cells that were not treated with bioactive glass were able to produce mineralized matrix but only in the presence of high concentrations of inorganic phosphate and dexamethasone. Mineralization was low on day 7 and moderate on day 14 of the culture. In contrast, the control bioactive glass (no metal dopant) was able to induce low levels of mineralization on day 7 in the absence of inorganic phosphate and dexamethasone and this mineralization increased by day 14. Copper and nickel-doped bioactive glass induced similar responses to that of the control bioactive glass. In culture wells treated with intermediate doses of dexamethasone and inorganic phosphate, vanadium, chromium, and nickel-doped bioactive glasses produced a more intensely stained matrix than the undoped bioactive glass. The effect of the Cr-doped bioactive glass was unexpected based on the viability data and represents an area of possible future research. Matrix deposition and mineralization was greatest in the MC-3T3 cultures grown in high concentrations of inorganic phosphate and dexamethasone in the presence of 1 mg/mL V or Cr-doped bioactive glass. Similar results were also obtained with the alkaline phosphatase specific activity assay.

Conclusions: Bioactive glass can indeed stimulate osteoblast activity and this is further enhanced by the presence of transition metal ions including vanadium, chromium, iron, and nickel. Further testing in this area will include assays of other osteoblast markers including osteocalcin and osteopontin expression. The most likely mechanism to explain these effects is the activation of metallothionein by the dissolution products of the bioactive glass. As metallothionein can be activated by many different ions, future research will include other bioactive glass compositions doped with transition metals such as cobalt and zinc.