Multivalent Presentation of Growth Factors by Cartilage Oligomeric Matrix Protein

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Introduction: The purpose of this work is to demonstrate that the presentation of growth factors (GFs) by COMP enhances the growth factor efficacy for osteochondral tissue engineering. Growth factors including bone morphogenetic protein (BMP) and transforming growth factor beta (TGFB) have tremendous clinical utility in forming bone and engineered cartilage tissues. It is common knowledge that these growth factors require binding to extracellular matrix (ECM) proteins for their full activity on cells. However, the mechanisms through which these GF/ECM binding interactions enhance growth-factor activity remain largely unknown. Cartilage Oligomeric Matrix Protein (COMP) is a homo-pentameric extracellular protein abundant in cartilage and bone. We recently discovered that COMP binds to BMPs and TGFB growth factors. Importantly, this binding enhances growth factor activities both in-vitro and in-vivo. COMP enhances the osteogenic activity of BMP2 in a rat model of spinal fusion, and COMP enhances the chondrogenic activity of TGFB1 for cartilage tissue engineering. Previous work by others established that many COMP binding interactions require zinc and manganese, two trace-elements that are absent in serum-free cell culture media. Here we explore the effects of these trace elements on osteochondral tissue engineering in the presence of COMP. We believe that enhancing COMP's ability to bind with the matrix by adding zinc may further enhance COMP's ability to present growth factor to receptors and enhance the chondrocyte phenotype.

Methods: Human cartilage samples were acquired from consented patients undergoing TKA or THA surgeries at our institution, with IRB approval. Chondrocytes from different donors were either extracted from cartilage by collagenous digest or supplied as clinical grade autologous cultured chondrocytes. Chondrocytes were expanded in monolayer to passage 3 maximum. Cell viability in the presence of zinc sulfate (0-320uM) was measured by WST-1 assay in monolayer cultures of chondrocytes after 2 days. Chondrogenic pellet culture was with $4x10^5$ cells per well of 96-well conical-bottom non-adherent culture plates, centrifuged 350xg for 5 min. Pellets were cultured for 3 weeks with media changes every other day in chondrogenic base media of 50 ug/ml ascorbic acid, 40 ug/ml L-Proline, 100 nm dexamethasone, 1% ITS, 15mM HEPES, and 10 ng/ml TGFB-1 and supplemented with zinc sulfate and/or COMP from 2-200uM or 0.1 – 15 µg/ml, respectively. After 3 weeks, pellets were weighed to obtain wet weight (ww) and imaged, and glycosaminoglycan (GAG) content measured by DMMB assay. Data from was analyzed with ANOVA with Tukey's HSD test. Human 293T cells were stably transfected to over-express recombinant human COMP with a C-terminal HisTag. COMP was extracted from the media by nickel-affinity chromatography, resulting in >95% purity by SDS-PAGE. Purified COMP was imaged with atomic force microscopy to visualize the expected pentameric structure.

Results: Atomic Force Microscopy revealed that the recombinant COMP was pure, and primarily in pentameric form (Figure 1). The addition of Zinc was non-toxic to monolayer chondrocytes, up to 320 uM (Figure 2A). In TGFB-induced chondrogenic pellet culture, zinc increased the pellets wet weight or GAG/ww ratio, with an optimal zinc concentration of 50 uM (Figure 2B). Above 50 uM zinc, the pellets were of inferior quality, soft and gelatinous (data not shown). We therefore used 50uM zinc for the remaining experiments. COMP also enhanced the wet weight of the pellet cultures, either alone or in combination with 50 uM zinc (Figure 3). The enhancing effect of COMP did not appear to be additive with that of zinc. Although COMP and zinc consistently enhanced TGFB-induced in-vitro chondrogenesis in all donors tested, there was variability between donors. In some donors the effects were most apparent in pellet size, wet weight, and total GAG content, whereas in other donors the effects were most apparent in the GAG/wet weight measurements. One possible explanation may be donor-dependent proliferation rates of the isolated chondrocytes, but this remains to be proven.

Discussion: We demonstrated that growth factor presented on COMP presents enhances the growth factor activity. COMP also other extracellular matrix elements such as chondroitin sulfate and collagen, and many of these interactions are cation-dependent. Perhaps these cation-dependent interactions are also with other ECM elements are important for the enhancing effect of COMP on growth factors that we observe. Zinc is an ion not typically present in the serum-free and chemically defined media used for tissue engineering. Therefore, the effects of zinc on chondrocyte pellet culture was determined. Previous studies demonstrate that zinc enables a more effective COMP-collagen interaction, which would help to tether COMP in close proximity to the cells while presenting TGFB-1 to the receptors. While the number of donors is limited in these preliminary experiments, these results highlight simple and novel additions to culture technique that may increase the efficacy of current orthopedic therapy and approaches the production of engineered osteochondral tissues.

Significance: COMP and divalent cations may have clinical utility to more effectively fill defects or maintain a better chondrocyte phenotype.

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Figure 1

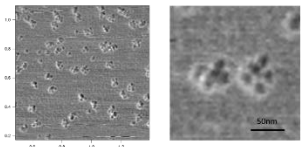

Figure 2

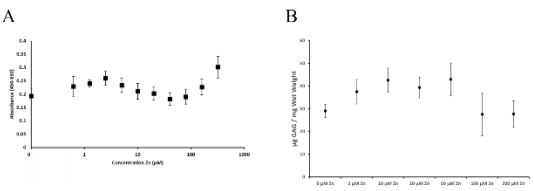

Figure 3