Silicon Ions Enhance Myogenic Differentiation in C2C12 Skeletal Muscle Cells

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The regeneration of bone and muscle tissue following musculoskeletal injuries is essential in sports medicine in order to restore function and prevent chronic musculoskeletal disorders related to physical inactivity. According to data collected by the American Academy of Orthopaedic Surgeons from 2012 to 2014, 8.3% (approximately 2.6 million people) of the adult population in the United States received treatment for musculoskeletal injuries, costing an aggregate total of $213 billion dollars. Recent regenerative musculoskeletal research suggests that the restoration of function and structure for normal physical activity is dependent on the synergy of regeneration processes found in bone and muscle tissue. Current treatments for severe musculoskeletal defects lack biocompatibility and rarely restore full function, so focus has shifted to regenerative biomaterials. Recent evidence indicates that bioactive gels and implants incorporating silicon (e.g. silicon ion, orthosilicate acid, amorphous silica) markedly increased osteogenesis *in vitro*, but little research has been conducted over the effect of these biomaterials on myogenesis. Establishing the existence of myogenic properties in silicon could lead to the development of a biomaterial that enhances the synergistic capacities of bone and muscle regeneration. **PURPOSE**: The purpose of this study was to investigate the effect of silicon ions on C2C12 skeletal muscle cells *in vitro*, in order to determine the regenerative viability of musculoskeletal gels and implants incorporating silicon. **METHODS**: In order to evaluate the effect of Silicon ions on myogenesis, *in vitro* cell culture studies were performed using C2C12 mouse myoblast cell lines. Cells were differentiated for four and seven days in media containing three different concentrations of silicon ions (0.1, 0.5, and 1.0 mM) and a silicon free control. Samples were immunohistochemically stained and imaged using a Zeiss fluorescent microscope. Cellsens software was used to determine total nuclei count and ImageJ was used to count the number of fused nuclei within myotubules. Rates of myogenic differentiation were determined based on fusion index, the percent of nuclei found within myotubes relative to the total nuclei. **RESULTS**: After four days there was a significant increase in fusion index ($p<0.001$) in the 0.1mM group (41 ± 3.4) compared to the control (31 ± 4.2) . After seven days all three silicon groups exhibited significantly higher fusion indices (0.1mM 60 ± 3.2, $p<0.001$; 0.5mM 57.8 ± 2.9, $p<0.01$; 1.0mM 54.8 ± 3.6 $p<0.05$) compared to the control (50 ± 2.4). Both four and seven day studies confirmed that the 0.1 Mm group had a markedly higher fusion index, indicating the highest rate of myogenic differentiation. **CONCLUSION**: Based on these results it can be concluded that silicon ions enhance myogenic differentiation. The myogenic potential of silicon ions exhibited by these results, combined with previously reported osteogenic effects, prompt further investigation into the potential of silicon-containing biomaterials to accelerate musculoskeletal regeneration, and decrease the risk of acute and chronic complications of injury.