# The Response of Progenitor Cells and Macrophages on a Bone Substitute to Biologically Relevant pH Levels

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**Statement of Purpose:** Delayed bone healing, or non-union, occurs in 5-10% of all fracture cases and in 20% of trauma fractures[1]. Increasingly, physicians are turning to synthetic bone substitutes because the gold standard, autograft, is limited in availability and require a second surgical intervention. Fracture healing is accompanied with a change from acidic to basic pH [2], and extensive work has evaluated cellular functionality of bone-relevant cells in a 2-dimensional setting at these varying pH levels[3]. Acidic environments have been shown to decrease the ability of osteogenic differentiation as well as increase the inflammatory response. Much less is understood about the increase above physiological pH during the fracture repair process. Our goal is to study the changes in pH to determine how they may affect progenitor cells, that are recruited to the site and commonly used in conjunction with bone substitutes, as well as inflammatory cells in their interaction with a commercially available calcium phosphate bone substitute.

**Methods:** Commercially available chronOS granules were heat sterilized at 200°C for 2 hours and placed into wells of a 24-well plate, 150mg of chronOS per well. Murine mesenchymal stem cells (D1, ATCC) and murine monocyte/macrophage cells (RAW 264.7, ATCC) were independently seeded onto the chronOS granules at a density of 1E6 and 1E5 cells per well, respectively. Cells were allowed to adhere overnight at incubator conditions, 37°C and 5% $CO_2$. To alter the pH, Dulbecco's Modified Eagle's Medium (DMEM) was supplemented with 15mM HEPES buffer and placed in incubator conditions to equilibrate. After equilibration, a specified amount of 3N HCl or 3N NaOH was added to each treatment to prepare DMEM aliquots with pH values of 7.0, 7.4, and 7.8; the pH values were verified using a modified colorimetric model. After 2 days of culture, the cells were lysed and analyzed for deoxyribonucleic (DNA), triglyceride, and alkaline phosphatase levels. Live/Dead imaging was used to assess cell attachment to chronOS and viability following treatment. All treatments were evaluated with a sample size of four and tested in duplicate for each assay. Statistical analyses were completed using Dunnett's Method to evaluate treatments in comparison to the physiologically normal pH of 7.4 with significance considered at a p-value<0.05.

**Results:** D1 cells produced a significantly smaller amount of alkaline phosphatase at a pH of 7.0 compared to that produced at a pH of 7.4, while no changes were documented in RAW 264.7 cell cultures. No pH-induced significant differences in D1 DNA amounts were noted for any treatment groups, but DNA levels at a pH of 7.0 were less than those at pH 7.4 (approaching significance). RAW 264.7 cells had significantly lower amounts of DNA at a pH of 7.0 compared to a pH of 7.4. Neither cell line cultures had any significant differences in triglyceride levels.

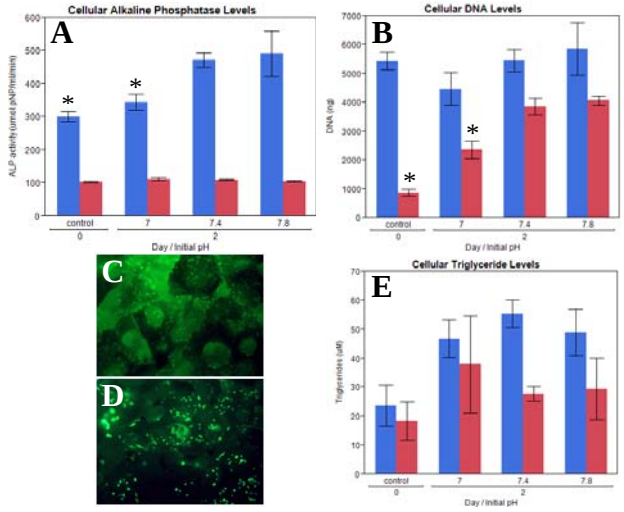


**Figure 1**. Blue: D1 cells; Red: RAW 264.7 cells *(A)* Alkaline phosphatase activity levels cells seeded on chronOS granules. *(B)* DNA levels of cells seeded on chronOS granules. *(C)* Live/Dead imaging of D1 cells attached to chronOS. *(D)* Live/Dead imaging of RAW 264.7 cells attached to chronOS. *(E)* Triglyceride levels of cells seeded on chronOS granules. Dunnett's Method was used to compare values to the day 2 pH level of 7.4 ($p<0.05$), i.e. the physiological control.

**Conclusions:** Preliminary results indicate that, similar to findings from 2D cultures, mesenchymal stem cells have decreased osteogenic activity at pH values lower than physiological levels, and have a smaller effect on cellular proliferation. Literature reports describe extensively that decreased pH increases the activity of macrophages, most commonly in their development into osteoclasts. These results indicate a decreased proliferation, possibly due to slowed cell proliferation and increased osteoclast differentiation. Further work is necessary to characterize the inflammatory activity of these cells and to consider the cross-talk between them.

**Acknowledgements:** Synthes Inc.; Hunter Endowment

**References:**

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