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Nanocalcium Sulfate and Collagen for Tissue Repair

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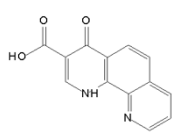
OBJECTIVES

- Attempt to study and replicate the tissue regeneration affects most commonly achieved by existing, but less accessible products used most often in contemporary clinical practice with a more accessible, affordable and biocompatible product that can help better meet the needs of patients with intellectual and developmental disabilities.
- Determine if nCS+Collagen is a viable option for common congenital defect repairs. (i.e. Cleft Lip/Cleft Palate)
- Determine if nCS can serve as a vehicle for 1,4-DPCA and release it in a time and concentration dependent manner
- Determine if nCS+1,4-DPCA supports human osteoblastic cell growth and differentiation



INTRODUCTION

Guided bone regeneration (GBR) using the principle of guided tissue regeneration (GTR) has been widely demonstrated to be a useful technique for enhancing hard and soft tissue healing in a variety of osseous and oral maxillofacial commonly observed in patients with intellectual and developmental disabilities. Placement of a biocompatible membrane to guide desirable cells such as osteoblasts to the appropriate site is a critical step in GBR. Optimization of the process includes the prevention of collapse of the membrane with bone grafting materials that also enhance the growth and differentiation of osteoblastic cells at the desired site. All of which can be difficult to achieve using traditional clinical methods considering the challenges many special needs patients may face, which include, but not to compliance issues, socioeconomic barriers, access/availability to specialized care, contributory systemic health factors, etc. The purpose of this in vitro study was to assess the ability of a nano-sized calcium sulfate product (nCS) to enhance the growth and differentiation of human osteoblastic cells on biocompatible collagen materials commonly used in GBR and GTR.

MATERIALS AND METHODS

Human Osteoblasts

- Obtained as frozen cultures and cultured under sterile conditions
- Cultured in alpha Minimum Essential Medium (MEM) and incubated at 37C and 5% CO_2

Nano Calcium Sulfate

- Formulated and patented by Dr. Dziak's lab
- Calcium sulfate is utilized as an osteoconductive scaffold that increases bone regeneration while maintaining space and preventing soft tissue invasion.
- Approach were taking is tissue engineering approach where calcium is working as a scaffold for the human osteoblasts
- It has been proven to be a safe, biocompatible material that can be mixed with fluids to yield a moldable paste that sets to form a scaffold that supports bone regeneration1-5.
- It is easily used as a delivery vehicle for growth factors, drugs and antibiotics****

MTT Assay: Cell Viability

- Measures cell viability via metabolicactivity
- Cells were incubated with varying concentrations of nCS loaded with 1,4-DPCA for 48 hours in a 96 well plate
- Media was removed from each well and MTT reagent and clear MEM-alpha was added
- The cells were allowed to incubate for another three hours, followed by the addition of DMSO
- The results are read at 540nm

Light Spectrometry Release Assay

Assay measured the amount of 1,4-DPCA released from nCS-DPCA disks incubated in 1-mL of Phosphate Buffered Saline (PBS) solution and overtime was measured at predetermined time intervals.

Evaluated the release kinetics of 1,4-DPCA from the nCS-DPCA disks overtime, the amount of of 1,4-DPCA present in each of collected samples were measured by spectrometry.

Preliminary Data

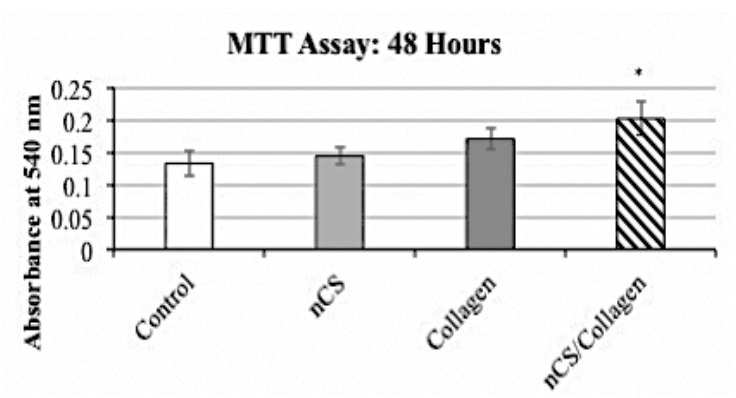


Figure 1. MTT activity in the nCS+Collagen group was higher than that of the control and nCS and Collagen alone groups; Values are the mean absorbance + standard deviation * indicates a significant increase in nCS+Collagen group compared with the other groups. n= 4; P<0.05 ANOVA.

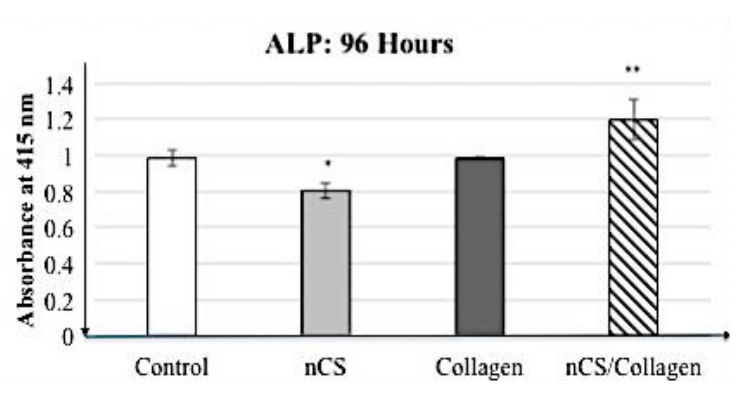


Figure 2. Alkaline phosphatase (ALP) activity in the nCS+Collagen group was higher than that in the control group (cells only in culture well) as well as nCS and Collagen alone groups. Values are the mean absorbance + standard deviation; * indicates a difference compared to controls at P<0.05. ** indicates a significant increase in ALP activity when comparing nCS+Collagen with the other groups. P<0.05 ANOVA; n=4 in all groups.

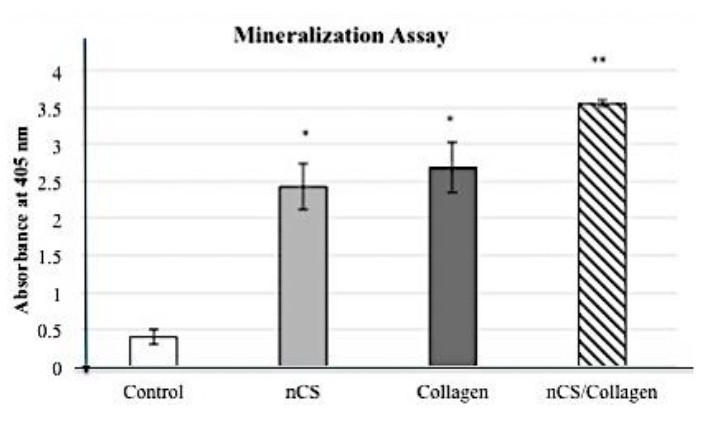


Figure 3. Cells were incubated for 21 days with the indicated materials. Mineralization activity in the nCS+Collagen group was approximately 7-fold higher than that in the control group. Values are the mean absorbance + standard deviation; * indicates a significant increase compared to control P<0.05. ** indicates a significant increase in mineralization when comparing nCS+Collagen with the other groups. P<0.05 (ANOVA); n=4 in all groups.

Results

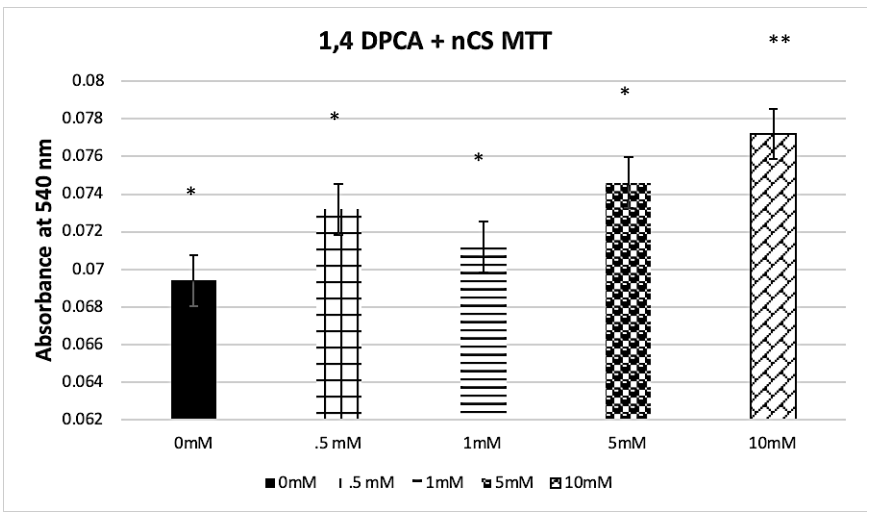


Figure 4. MTT activity across increasing concentrations of 1,4-DPCA illustrate the 10mM of the aqueous solution loaded into nCS had an increased level of mitotic activity when compared to the control. Values are the mean absorbance + standard deviation; * indicates a difference compared to controls at P<0.05. ** indicates a significant increase in ALP activity when comparing nCS+Collagen with the other groups. P<0.05 ANOVA; n=5 in all groups.

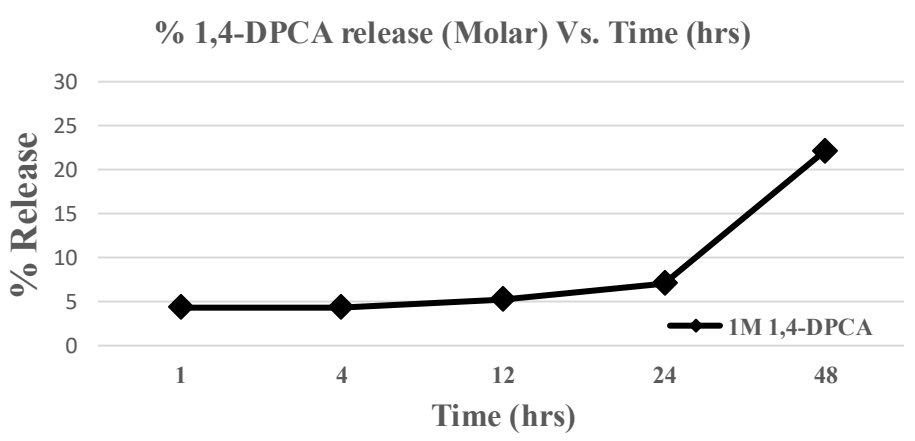


Figure 5. Drug release study of 1,4-DPCA. Each time point represents the percentage of 1.4-DPCA released from an nCS pellet at four different time points, 1, 4, 12, 24 and 48 hours, in phosphate buffer solution. The data series represents 1M concentration of aqueous 1,4-DPCA from which nCS was loaded.

CONCLUSIONS

nCS is a biocompatible material which has the potential when combined with the drug 1,4-dihydrophenonthrolin-4-1 - 3-carboxylic acid (1,4-DPCA) to act as an effective drug delivery vehicle and does not have any toxic effects on human osteoblastic cell growth.

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