Test of BMP2-Induced Differentiation

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Summary

Cells were either transiently or continuously exposed to human recombinant BMP2 protein at either 100 ng/mL or 300 ng/mL for four days to assay for differentiation. Micrographs were taken on day 5 for analysis of morphology, and ALP assay was performed on day 5 to assay for ALP activity as a marker of osteogenesis.

Background

BMP2 is a well estabished pro-osteogenic morphogen shown to induce osteogenesis in a variety of cell types in literature. Upon binding BMP2, the BMPRI receptor activates a downstream signaling cascade involving Smad proteins, which results in activation of the Runt-related transcription factor 2 (RUNX2). RUNX2 is the main transcription factor controlling osteoblast differentiation; transfection of RUNX2 induced osteoblastic differentiation of fibroblasts, which do not normally differentiate into osteoblasts.

ALP is a routine assay used to determine differentiation. It measures activity of an osteoblast-specific protein, alkaline phosphatase. This enzyme increases the local concentration of phosphate, which aids formation of the the hydroxyapatite ion that underlies mineralization in bone. The assay was used to determine effectiveness of human recombinant BMP2 dissolved in medium in inducing osteoblastic differentiation in C2C12, C3HT10 1/2, and HEK cells.

Methods

Schematic of Cell Seeding

(-)	(-)	(-)	(-)		
(-)	(-)	300 ng/mL	300 ng/mL		
100 ng/mL	100 ng/mL				
300 ng/mL	300 ng/mL				
C2C12	C3HT101/2	HEK	C3HT101/2	C2C12	C3HT101/2
+ BMP2	**+ BMP2**	**+ BMP2**	**+ BMP2 (Transient)**	**Discard**	**Discard**

General Notes

- Human Recombinant BMP2 harvested from E. Coli from Insight Genomics, VA
  Reconstituted in 20 mM acetic acid in H2O to 100 ug/mL, total 90 uL
- At end point of BMP2 stimulation ALP assay will be performed, tentative date Tuesday 7/6

Cell Culture Schematics

Key

Color	Meaning
	Media change only
	Add BMP2 accordingly
	No Action Required

Friday 7/2

(-)	(-)	(-)	(-)		
(-)	(-)	300 ng/mL	300 ng/mL		
100 ng/mL	100 ng/mL				
300 ng/mL	300 ng/mL				
C2C12	C3HT101/2	HEK	C3HT101/2	C2C12	C3HT101/2
+ BMP2	**+ BMP2**	**+ BMP2**	**+ BMP2 (Transient)**	**Discard**	**Discard**

Concentration (ng/mL)	# Wells	a-MEM	Stock (100ng/uL)
300	2	1 mL	3 uL
100	1	.5 mL	.5 uL

Notes

- Assume concentrations were 300 ng/mL and not 300 ng/uL
- In order to ensure solubility, BMP2 was vortexed for 5 min, centrifuged at 18,000g for 10 minutes to make sure no precipitate formed before it was added to the medium
- Added .4 mL total volume to conserve protein

Concentration (ng/mL)	# Wells	D-MEM	Stock (100ng/uL)
300	2	1 mL	3 uL
100	1	.5 mL	.5 uL

Saturday 7/3

		(-)			
		300 ng/mL			
C2C12	C3HT101/2	HEK	C3HT101/2	C2C12	C3HT101/2
+ BMP2	**+ BMP2**	**+ BMP2**	**+ BMP2 (Transient)**	**Discard**	**Discard**

Concentration (ng/mL)	# Wells	D-MEM	Stock (100ng/uL)
300	1	.5 mL	1.5 uL

Sunnday 7/4

(-)	(-)	(-)	(-)		
(-)	(-)	300 ng/mL			
100 ng/mL	100 ng/mL				
300 ng/mL	300 ng/mL				
C2C12	C3HT101/2	HEK	C3HT101/2	C2C12	C3HT101/2

+ BMP2	+ BMP2	+ BMP2	+ BMP2 (Transient)	Discard	Discard

Concentration (ng/mL)	# Wells	a-MEM	Stock (100ng/uL)
300	1	.5 mL	1.5 uL
100	1	.5 mL	.5 uL

Concentration (ng/mL)	# Wells	D-MEM	Stock (100ng/uL)
300	2	1 mL	3 uL
100	1	.5 mL	.5 uL

Monday 7/5

		(-)			
		300 ng/mL			
C2C12	C3HT101/2	HEK	C3HT101/2	C2C12	C3HT101/2
+ BMP2	**+ BMP2**	**+ BMP2**	**+ BMP2 (Transient)**	**Discard**	**Discard**

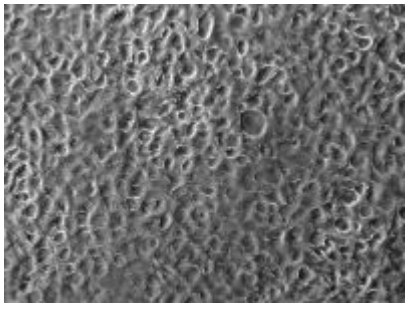
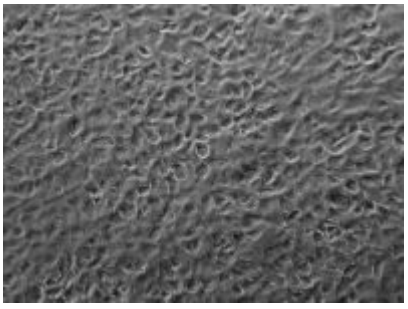
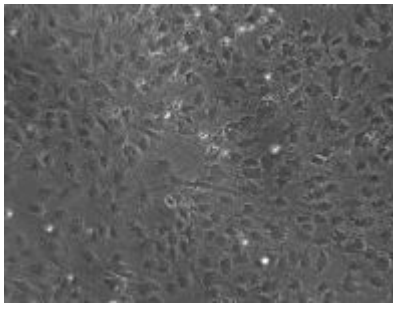
Concentration (ng/mL)	# Wells	D-MEM	Stock (100ng/uL)
300	1	.5 mL	1.5 uL

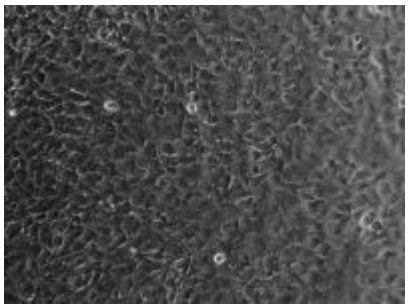
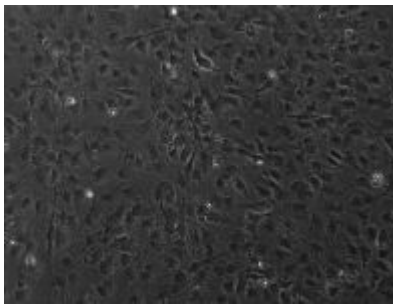
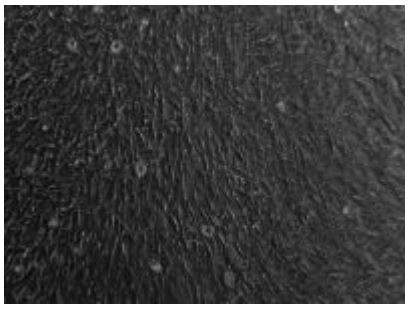
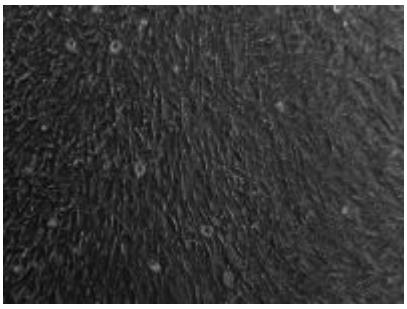
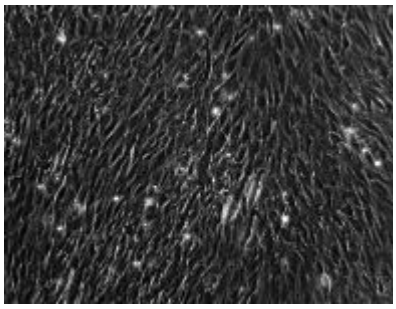
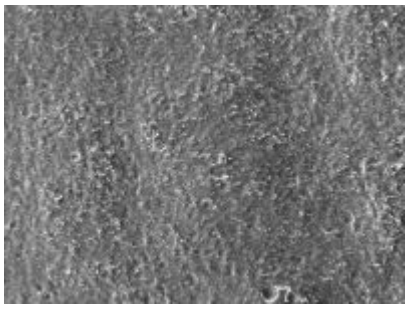
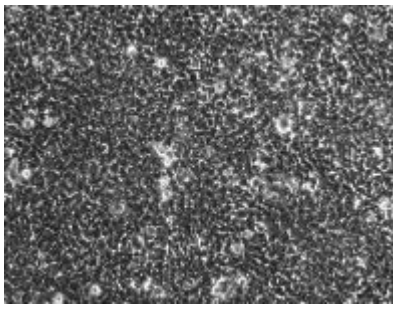
Tuesday 7/6

ALP Assay
Micrographs

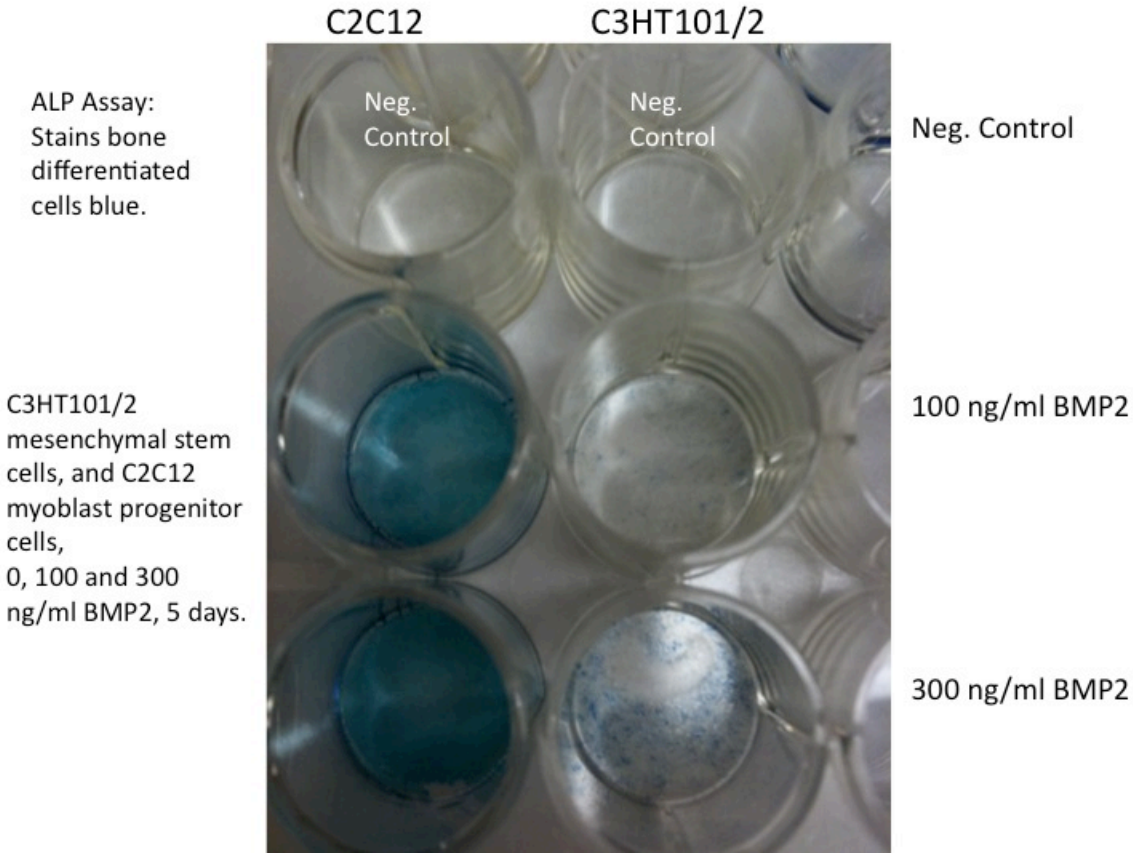
Results

Morphology

Cells	300 ng/mL	100 ng/mL	0 ng/mL
C3HT101/2			

C3HT10 1/2 (Transient)		-	
C2C12			
HEK		-	

ALP Assay



Results:

C2C12 displayed a strong increase in alkaline phosphatase activity in cells treated with 100ng/ml and 300ng/ml BMP2, when compared with untreated controls. The C3HT101/2 mesenchymal stem cells exhibited a significantly weaker, but still visible upregulation of ALP in cells treated with BMP2.
HEK and transiently stimulated C3HT101/2 did not display visible upregulation of ALP activity.

Conclusion

C2C12 cells and C3HT101/2 cells both are able to differentiate into osteoblasts in response to BMP2 stimulation as indicated in the literature. Interestingly, C2C12 cells dfferentiated in greater numbers and at lower BMP2 concentrations than the mesenchymal stem cells. If the Alizarin Red assay shows that mineralization efficiency follows the same pattern, C2C12 cells will be our best choice for actuator cells for the final system. Finally, since 100 ng /mL BMP2 are enough to differentiate them efficiently, this sets the target for BMP2 secretion by mechanically stimulated HEK cells.