

## Investigating UBM Degradation Products as a Possible Therapeutic Treatment for Regenerative Medicine using a P19 Cell Model

G. Iwaoka<sup>1</sup>, C. Queenan<sup>2</sup>, A. Calabro<sup>2</sup>, D. Leonardi<sup>1</sup>

<sup>1</sup>Bergen County Academies, Cell Biology Lab, 200 Hackensack Avenue, Hackensack, NJ 07601

<sup>2</sup>Bergen County Academies, Nano-Structural Imaging Lab, 200 Hackensack Avenue, Hackensack, NJ 07601

*In vivo*, degradation products of extracellular matrix are crucial in the wound healing process where matrix metalloproteinases (MMPs) and protease inhibitors, known as tissue inhibitors of metalloproteinases (TIMPs), are responsible for the release and modulation of growth factors derived from degraded ECM. This modulation contributes to the cell proliferation, migration, and differentiation that occur in wound healing [1]. Urinary Bladder Matrix (UBM) is the decellularized product of the extracellular matrix of pig bladder which has been previously shown to promote soft tissue regeneration [1,2]. However, the mechanism of action of UBM is poorly understood [3,4].

Mouse embryonic carcinoma cells derived from murine teratocarcinomas (P19 cells) were introduced as a model cell line to investigate these events *in vitro* [5]. Three aspects of wound healing were compared: differentiation, proliferation, and migration. Two UBM preparations were used: UBM enzymatically digested with pepsin to simulate degradation and UBM eluate, in which larger protein components of the extracellular matrix were removed by centrifugation, with growth factors or small fragments of collagen remaining in solution.

To analyze the effect of pepsin-digested UBM on cell differentiation, pluripotent P19 cells were treated with 75 µg/mL dry weight UBM. Three unique cell types were established after treatment: undifferentiated P19 cells (P19SSEA-1), UBM-differentiated P19 cells (P19UBM), and spontaneously-differentiated P19 cells (P19GFAP). These cell types were characterized by light microscopy (Figure 1) and immunocytochemistry (Figure 2). P19SSEA-1 cells formed aggregates, lacked projections and were positive for the pluripotent marker SSEA-1. P19GFAP cells showed dendrite-like structures, similar to astrocytes, and expressed the differentiation marker GFAP. P19UBM cells showed multiple forms of differentiation; morphologically the cells appeared either branched or compacted and expressed a range of differentiation markers (MAP2, O1, and GFAP).

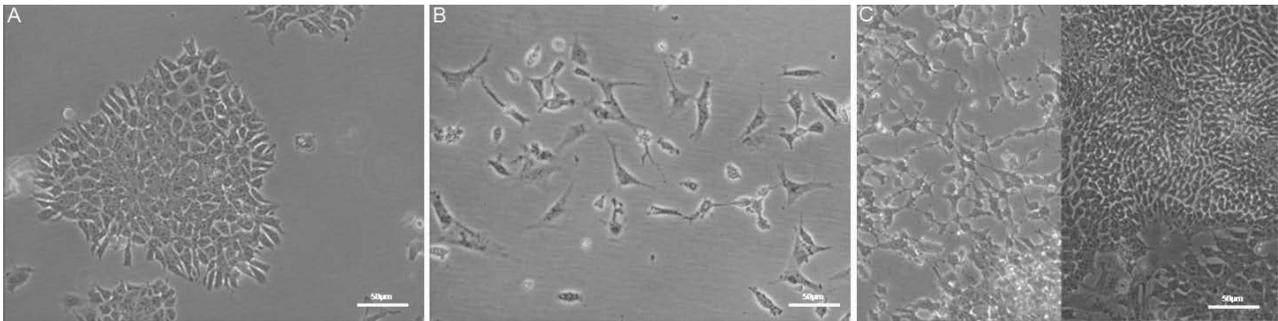
Proliferation was measured using a MTS assay after treatment with 25, 50, 75 and 100 µg/mL of pepsin-digested UBM and treatment with UBM eluate. The cells showed an increase in viability across increasing concentrations of pepsin-digested UBM, except P19UBM cells which showed reduced viability at lower concentrations (25, 50µg/mL). All cell types had decreased viability after treatment with UBM eluate. Results of proliferation assays were all statistically significant ( $p < 0.05$ ).

Migration (chemotaxis) toward UBM (200, 300, 400, 500µg/mL) was measured using a 96 well plate migration chamber and results showed an increase in migration of up to 2053% in P19SSEA-1 cells and 414% in P19GFAP cells, while P19UBM cells demonstrated reduced chemotaxis as compared to control. These results were also statistically significant ( $p < 0.05$ ).

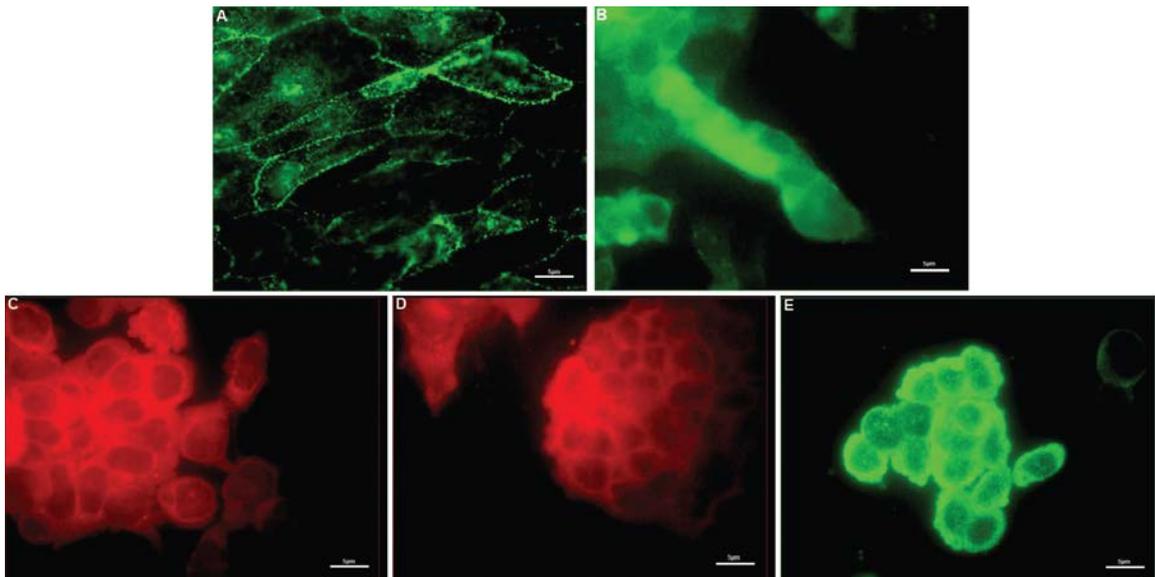
Results from this experiment suggest that pepsin-digested UBM induced differentiation in P19 cells. Because results from the proliferation and migration experiments varied based on the type of UBM treatment, pepsin-digested or eluate, it is suggested that different components of UBM are responsible for inducing proliferation/migration at different stages of differentiation.

#### References:

- [1] E. Brennan, et al., *Tissue Engineering* 12(10) (2006) 2949-2955.
- [2] T. Giblert, et al., *Biomaterials* 26(12) (2005) 143-1435.
- [3] S. Badylak, *ExpHematol* 29(11) (2001) 1310-1318.
- [4] T. Zantop, et al., *JOrthop Res* 24(6) (2006) 1299-309.
- [5] M. Van der Heyden, et al. *Cardiovascular Research* 58(2) (2003) 410-422.
- [6] The authors would like to thank Dr. Stephen Badylak and his team at the McGowan Institute of Regenerative Medicine for supplying protocols and samples of digested UBM pepsin; Mrs. Rachel Stott, Dr. Donald DeWitt, and Dr. Robert Pergolizzi and the administration of the Bergen County Technical School District and the Bergen County Academies for their continued support.



**Figure 1:** Phase contrast images of P19 differentiation. A) Undifferentiated P19 cells. B) P19 cells after spontaneous differentiation. C) P19 cells after UBM treatment. Scale bars = 50µm.



**Figure 2:** Fluorescent images from immunocytochemistry experiment. A) Untreated P19 cells expressing only pluripotent SSEA-1 marker. B) Spontaneously differentiated P19 cells expressing only GFAP marker (non-pluripotent). P19 cells treated with UBM expressed the markers GFAP (C), O1 (D), and MAP2 (E), showing differentiation along several cell types. Scale bars = 5µm.