DEVELOPMENT OF A REGENERATIVE WOUND DRESSING FOR IMPROVED HEALING IN SPACE

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Introduction

ACell, Inc. currently markets multiple configurations of its Urinary Bladder Matrix (UBM-ECM) product, called MatriStem®. This material is derived from the decellularized basement membrane and tunica propria layers of the porcine bladder and consists of extracellular matrix proteins including collagens, glycosaminoglycans, and growth factors, which provide both a structural and biological framework for tissue healing [1,2].

Although the mechanism of action behind ECM-mediated constructive remodeling is not yet fully understood, it has been hypothesized that these ECM-derived biomaterials act as *in situ* bioactive regenerative templates, serving as substrates for progenitor cell infiltration and differentiation. The ECM biomaterial can be considered a concentrated version of the body's own natural scaffold that occurs after injury. The body is hypothesized to act as a bioreactor, providing additional site-specific biomechanical and biochemical cues that guide cell differentiation. Several studies have demonstrated the chemo-attractive effects of UBM-ECM biomaterial breakdown products on progenitor cells.

Impact for Human Space Flight

Lacerations and abrasions are common in space flight due to restricted quarters, high activity levels and interaction of the cutaneous tissues with Extravehicular Mobility Units (EMU). Wound healing of cutaneous tissue is a complex, orchestrated process involving the interaction of soluble factors, extracellular matrix (ECM), inflammatory cells, and tissue-specific cells [2]. In space, conditions of microgravity and low oxygen tension affect the normal wound healing process. Specifically, conditions of microgravity have been shown to decrease the cellular response to growth factors, and reduced oxygen tension is known to delay healing and increase protease levels.

Although existing MatriStem products have the potential to improve healing in space, current dressing configurations (Figure 1) would be difficult to administer in low gravity and maintain position under high activity levels. In June 2012, ACell, Inc. received a Space Medicine and Related Technologies Commercialization Assistance Program (SMARTCAP) award from National Space Biomedical Research Institute (NSBRI) to develop a novel MatriStem UBM gel formulation which can be easily administered to wounds in space. The development of a novel gel formulation of MatriStem UBM technology that preserves the bioactivity and vulnerary properties of the biomaterial would provide astronauts with a powerful new tool to combat lacerations and abrasions incurred during flight missions. Promising prototypes have been developed and are undergoing *in vitro* screening to identify the most promising candidates for testing *in vivo*. A novel model of ischemic wound healing in rats has been developed which will be used to test the *in vivo* efficacy of the gel prototypes.

Figure 1: MatriStem single layer lyophilized sheet and powder

MatriStem® Gel Prototype

Various prototypes have been developed and are currently undergoing *in vitro* screening. Gels are created using UBM powder solubilized in NaOH basic conditions. All gels are neutralized using HCl following solubilization and checked for pH. These gels are then frozen and lyophilized to create a powder form of the gel. The powder is then reconstituted using water within two 3mL syringes. One syringe contains the lyophilized gel, the other water, and they are mixed together via a connecter between the two syringes. Various concentrations of UBM in NaOH have been tested for handling properties to determine their ability to be applied using the two syringe system. The final consistency of all gels is foam-like, and each one adheres to the surface it is applied to without dripping (Figure 2).

Gels were created using various concentrations of MatriStem powder (0.5-11% w/v) and molarity of NaOH (0.1 and1.0M). UBM was solubilized for various time periods (1-48 hours) in its respective concentration of UBM and NaOH at 4.0°C. In order to test whether the growth factors FGF-2 & VEGF in UBM could refold after solubilization, gels were also made using various dwell periods (1-48 hours) following neutralization. All of these candidates are currently being tested *in vitro* for growth factor content (FGF-2, VEGF, and CTGF), cell proliferation, chemoattractive properties, and growth factor viability via neuronal cell differention.

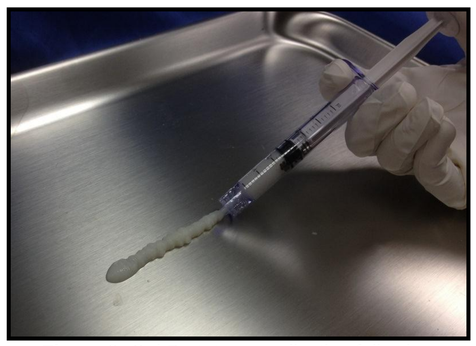

Figure 2: Gel Prototype application following reconstitution (7% w/v, 100mM NaOH)

Characterization

To date, studies have demonstrated that prototype gels have been able to retain measurable amounts of both FGF-2 and VEGF cytokines. Table 1 compares the FGF-2 and VEGF concentrations of currently marketed MatriStem powder, the baseline powder that all gels were made from, and the average of all gel configurations following NaOH solubilization [3]. Data for FGF-2 and VEGF content following solubilization for each gel structure is shown in Figures 3A and 3B respectively. FGF-2 content of the gels is clearly being knocked down by the basic conditions; however the VEGF levels may actually be increasing due to growth factor adherence within the matrix. Parallel studies on FGF-2 and VEGF knockdown have confirmed that treatment of porcine urinary bladders with various concentrations of NaOH are unable to knockdown the presence of VEGF in the material. Lower concentration gels showed similar trends to Figure 3A and 3B.

Table 1: FGF-2 and VEGF concentration comparisons

	FGF-2 Concentration	VEGF Concentration
MatriStem Powder	107.22±7.99 pg/mg	1.951±0.25 pg/mg
Baseline Gel Powder	178.43±18.14 pg/mg	20.57±0.92 pg/mg
MatriStem Gel	10.01±1.06 pg/mg	33.59±3.53 pg/mg

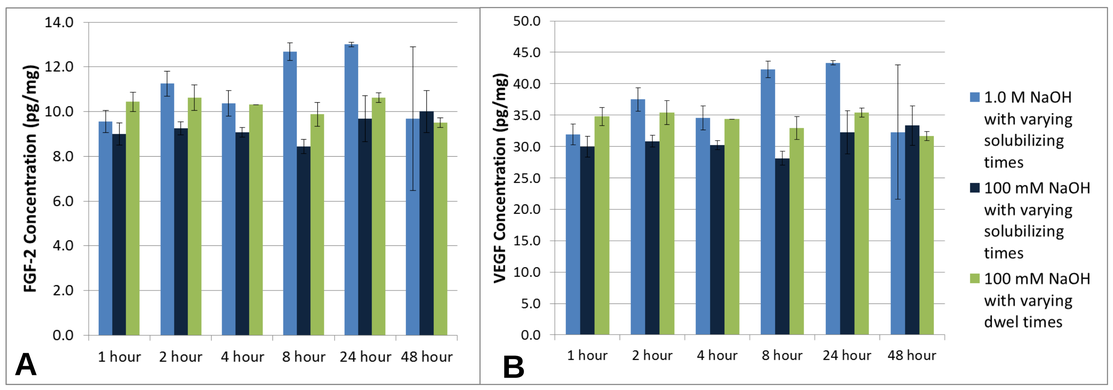

Figure 3: FGF-2 (A) and VEGF (B) concentrations of multiple gel prototypes. Note shared legend.

All prototypes tested retained similar amounts of both FGF-2 and VEGF. Surprisingly, there seems to be an increase in the release of both cytokines with 1.0M NaOH and longer digestion periods (8, 24 hours). Based on these results, the concentration of NaOH may not be relevant when deciding which gel prototype to pursue. However, based on the consistency and manufacturability of the gels, the ideal concentration of base will be considered 0.1M NaOH due to resulting NaCl concentration in the gel. The ideal concentration of UBM will be decided following more rigorous *in vitro* screening, including cell proliferation and chemotaxis assays.

In Vivo Screening

Following completion of *in vitro* testing, prototype candidates will be tested for efficacy using a novel model of ischemic wound healing developed in collaboration with Bridge Preclinical Testing Services (San Antonio, TX). Ischemia of the tissue is achieved by creating a bipedical flap and introducing a sterile silicone sheet underneath the flap to prevent healing. Model development has led to significantly delayed healing of ischemic wounds compared to control wounds (Figure 4).

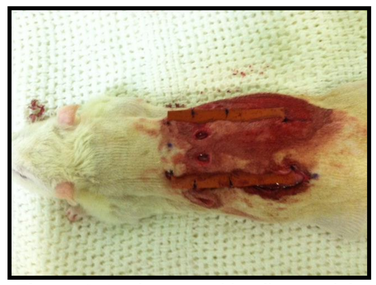

Figure 4: *In vivo* wound healing model.

Conclusions

MatriStem gel prototypes have shown promising results during *in vitro* screening. Following further *in vitro* analysis, gel candidates will be chosen to be tested *in vivo* in a novel ischemic wound study. The development of a gel formulation of MatriStem powder would allow astronauts to treat lacerations and abrasions during missions without the complications or restriction of current products. MatriStem gel will also be applicable to patients treated on earth, and will provide a novel product for wound repair.

References

1. Kentner K.A. et al (2012) Differential release of growth factor from MatriStem® urinary bladder matrix (UBM) products. Society for Biomaterials Fall Symposium, New Orleans LA.
2. Gilbert T.W. et al (2005) Production and characterization of ECM powder: Implications for tissue engineering applications. Biomaterials 26,1431–1435.
3. Kentner K.A. & Janis A.D. (2011) Quantification of FGF-2, VEGF, & GAGs in MatriStem MicroMatrix UBM Biomaterial. BMES Fall Meeting, Hartford, CT.

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