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PACT Update: Cell Therapy for Regeneration of the Vocal Fold

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Voice disorders are the most common communication disorder across the lifespan[1]. The voice is a primary mode of communication, is often tied to personal identity, and is paramount to the livelihood of millions of individuals. Consequently, voice disorders are not "cosmetic". On the contrary, impaired voice production holds significant implications for individual health and wellness, social and occupational function; and societal productivity[2,3]. Conservative estimates suggest that 3 to 9% of the general population has some type of voice abnormality[4,5] at any given moment in time and that 29% of the general population will have a voice disorder at least once in their life[4]. Vocal fold scarring, a specific voice disorder, is associated with fibrosis of the extracellular matrix (ECM) of the vocal fold which causes a significant increase in the stiffness and viscosity of this highly specialized tissue[6]. Treatment outcomes for patients with vocal fold scarring, an ECM injury or loss, remain largely ineffective despite the substantial remediative efforts that have been undertaken to date, see reviews[7,8]. Accordingly, there is a clinical need for the development of advanced treatments that appreciate the necessary remodeling of the ECM structure and biomechanical properties of the vocal folds. The long-term aim of our work is to develop injectable products that induce tissue regeneration to address vocal fold scarring. Our therapeutic approach includes using a cellular product consisting of either cells alone or in combination with a hydrogel matrix. Products that could be utilized to treat scarring of the vocal folds would ideally be non-immunogenic, non-toxic, non-inflammatory and easily injectable. The development of innovative treatment paradigms for vocal fold scarring has been hampered by lack of knowledge regarding cell sourcing and implementation of a combination product approach. Cell sourcing and cell characterization were recently recognized as critically important research areas that need attention if translational therapeutics are to progress[9]. While vocal fold fibroblasts (hVFF) regenerate ECM of the vocal fold, a native supply of healthy, clinical-grade, allogeneic supply of hVFF is not available for therapeutic development due to the impractical issues associated with sourcing of normal vocal fold tissue from live donors.

Over the past ten years, promising reports of BM MSC-based therapies for regeneration of scarred vocal fold tissue in animal models have been published[10-12]. A scientific rationale for BM MSC-mediated vocal fold scar attenuation is related to the similarity between hVFF and BM MSCs. Our group has previously demonstrated similar cell surface markers, immunophenotype, and differentiation potential between hVFFs and BM MSCs[13]. Specifically, two hVFF primary cell lines (p59 and p21) expressed MSC markers CD73, CD90 and CD105, and were negative for CD14, CD34 and CD45. The hVFFs were able to differentiate toward osteogenic, adipogenic and chrondrogenic lineages and showed the same immunological phenotypes as BM MSCs.

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The similarity between these two cell types provides support for the investigative use of BM MSCs in the vocal fold.

Our combination cell/gel therapy product includes BM MSCs and the use of a hyaluronic acid (HA) hydrogel, HyStem-VF, produced by Biotime, which has undergone rigorous biomechanical, biocompatibility, safety and toxicity testing *in vitro* and *in vivo* over the past ten years by our laboratory. Most relevant to this therapeutic approach, it has been documented that the restorative effects of BM MSCs can be amplified when delivered to scarred vocal fold tissue within an appropriate scaffold such as HyStem-VF. The synergistic effects of this combination therapy are promising. One underlying mechanism supporting the anti-inflammatory profile of this therapeutic is macrophage activation. It was recently reported that macrophages cultured with BM MSCs/HyStem-VF *in vitro* are more likely to have an anti-inflammatory immunophenotype (lower expression of CD16 and HLA-DR and higher expression of CD206) than macrophages cultured on tissue culture plastic or those cultured on HyStem-VF without BM MSCs[13].

Work in collaboration with the NHLBI Production Assistance for Cellular Therapies (PACT) group at the University of Wisconsin - Madison (Contract No. HHSN268201000010C) has included development of final product formulation, assay development, and delivery methods to support the translation of this technology from the research environment into the clinic. Additional funding for this work has been provided by NIH NIDCD R01 4336. The MSC Master Cell Banks (MCBs) Passage 2 (P2) were derived from bone marrow that was acquired from normal healthy donors that underwent full donor screening in compliance with 21 CFR 1271. The MSCs were isolated from a bone marrow aspirate MNC fraction and were expanded in a clean room manufacturing facility (Waisman Biomanufacturing) with cGMP documentation and quality control (QC) testing. The MSC MCBs have undergone full cGMP compliant QC testing and are currently stored at Waisman Biomanufacturing. The manufacturing process consisted of thawing and expanding several vials of MCB (P2) to the final product (P5), formulating the MSCs in appropriate buffer/cryoprotectant, and cryopreserving clinical-grade material in vials. Quality Control assays were developed to support in-process and final product lot release testing.

In an effort to create an ‘off the shelf’ product for commercial use, product formulation and delivery have been optimized and specifications developed so that the treatment is standard across various conditions/subjects/clinical sites. Assays have determined a favorable protocol for consistent cell mixing and uniform dispersion throughout the gel and identified an ideal gelation time for a clinically relevant number of cells within HyStem-VF or in isolation (Figure 1). Because of the unique shear thinning qualities of HyStem-VF, it can be easily passed through a needle. This shear thinning effect had an unknown effect of the viability of the BM MSC. Typically, 24-28 gauge needles are utilized in surgery to the vocal folds. In addition, the current formulation and packaging that is used for MSCs that are delivered by intravenous infusion had to be modified to provide a formulation with a higher cell concentration that will be cryopreserved in an appropriate vial for a small volume injectable. Our results indicate that for hydrogel-induced cell therapy, final product, containing $1x10^7$ cells/mL and less than 0.5%

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DMSO final concentration may provide exceptional cell viability for a regenerative medicine application (≥ 80% viability) (Figure 2). Further, 25G and 27G needle-induced physical force significantly decreased frozen cell survival rate to 83% compared to the force generated using a pipette (Figure 3).

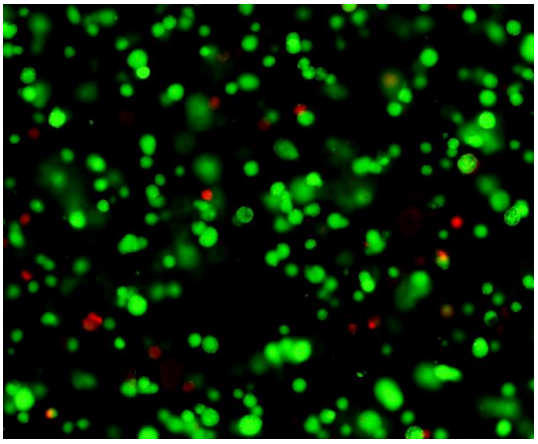
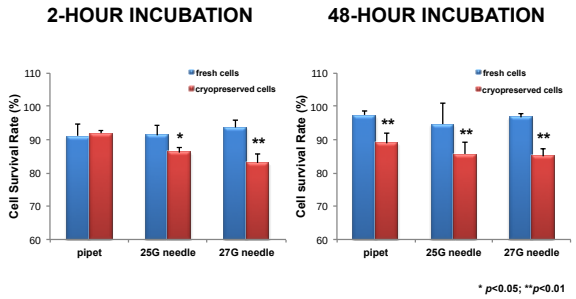


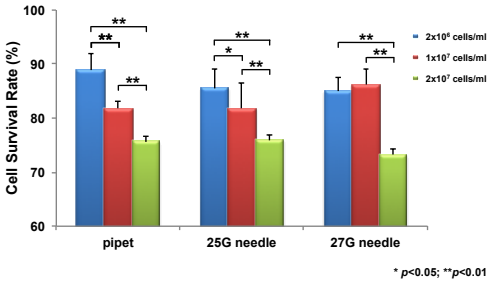
Figure 1: Frozen Clinical Grade BM MSC viability in HyStem-VF after 2 hour incubation. BM MSCs are dispersed throughout the gel. Double staining Live/Dead Viability/Cytotoxicity assay (Calcein-AM and EthD-1), (Green=live cells, Red=dead cells).

Figure 2: Cell viability by different cell delivery methods on fresh and cryopreserved cells



The development and refinement of appropriate formulation, packaging, and manufacturing methods was performed at Waisman Biomanufacturing. Documentation of all production components was completed in accordance with current Good Manufacturing Practice (cGMP). Final dosages formulation included cryopreserved human clinical doses using qualified materials and reagents to support human clinical studies. A total of 164 vials of BM MSC containing two dosages were manufactured and frozen for use.

Figure 3: Effect of Cell Density and delivery methods on Cryopreserved Cell Viability



Lastly, we are presently developing and testing potency assays that represent surrogate markers for the relevant paracrine activity of the MSC in HyStem-VF. This is essential as it will ensure consistent biological quality of the final clinical grade material. Identification of surrogate markers for the relevant anti-inflammatory, immunomodulatory and paracrine activity of the MSC will ensure consistent quality of clinical grade material; the markers will be based on the attributes of the product that have been linked to treatment efficacy in pre-clinical studies. We

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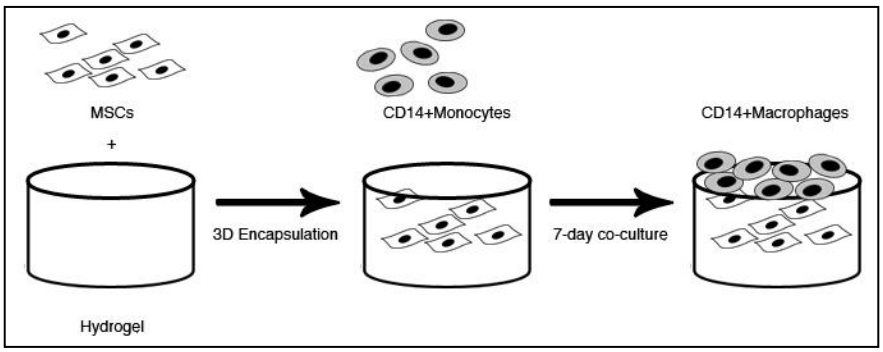

Figure 4: Schematic drawing of the co-culture system to be developed for the potency assay. BM MSC will be encapsulated in 3D HyStem-VF. Purified CD14 + monocytes will be added to the surface of the MSC-gel construct (1 x 10^6 cells) and maintained in culture for 7 days to generate macrophages.

will optimize a unique three dimensional (3D) co-culture system with MSCs encapsulated in the HyStem-VF hydrogel and macrophages derived from peripheral blood CD14+ monocytes plated in direct contact with the MSC-gel construct[13] to investigate the surface marker expression of monocyte-derived macrophages vital to tissue healing and inflammation (Figure 4). Following a seven day culture, media including non-adherent cells will be aspirated from each well. Previous work completed by our lab showed that monocyte-derived macrophages differentiated in the presence of this MSC-hydrogel construct will exhibit an anti-inflammatory phenotype as measured by an increase in the expression of the surface marker CD206 on the macrophages. Further potency will be measured by the ECM and cytokine production as measured by gene expression from the BM MSC seeded in the hydrogel. This critical development effort is necessary to perform pre-clinical safety studies using clinical-grade material to support an IND application which is required to translate our work into a human Phase I clinical trials for patients with vocal fold scarring.

While our research questions specifically address the clinical problem of vocal fold defects, we expect that our research and resultant findings will be valuable to others investigating tissue engineering of connective tissue in other physiological systems. Because we are studying the problem of scarring and the permanent restoration of appropriate tissue biomechanics, our BM MSC construct tissue engineering approach will be applicable to a wide range of medical fields. More specifically our cell production manufacturing, processing and delivery method refinement will be of interest to a large number of investigators who anticipate utilization of MSC in similar tissue engineering constructs including those whose areas are under the auspice of the National Heart, Lung, and Blood Institute.

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