**1University of Virginia, Charlottesville, VA, USA, 2Drexel University, Philadelphia, PA, USA.**

**Purpose:** The use of acellular dermal matrices (ADM) in implant-based breast reconstruction has grown significantly since the first reports in 2005. Success of ADM relies on its rapid integration into this host, which is reliant on cellular and vascular ingrowth. Macrophages are major regulators of tissue vascularization, and transition from pro-inflammatory M1, which initiate angiogenesis, to a phenotype referred to as M2, which is associated with stabilization of blood vessels and resolution of tissue healing. Both M1 and M2 macrophages in the proper order are required for tissue vascularization. In this study, we investigated incorporating immunomodulatory cytokines that promote the M1 and M2 macrophage phenotypes in order to improved ADM biointegration. We hypothesized that short-term release of pro-inflammatory, M1-promoting interferon-gamma (IFNg) or sustained release of the M2-promoting cytokine interleukin-4 (IL4) would control the dynamics of inflammation within the wound, leading to accelerated vascularization and collagen deposition in the ADM, thereby decreasing complications and improving outcomes.

**Methods:** Low (375ng) or high (750ng) doses of IFNg was adsorbed to ADM or to porous collagen scaffolds. Additionally, IL4 (5ug) was adsorbed onto ADM samples. Samples were then washed and the amount of unadsorbed protein was measured. Release studies were also performed to determine the elution profiles. Next, a mouse dorsal skinfold window chamber model was used to determine the effects of IL4 treated ADM on biointegration (n=8 IL4 treated ADM, n=8 untreated ADM). Real-time evaluations of the vascular integration and oxygen saturation within the ADM were made through repeated use of novel oxygen sensing nanoparticles over 21 days. At the terminal time point, macrophage function (M2:M1 ratio), and vascular ingrowth (CD31) were evaluated by immunohistochemistry.

**Results:** Surprisingly, ADM samples released high levels of cytokine for up to 11 days in vitro, unlike porous collagen scaffold controls, which released most of the cytokine within 2 hours. The adsorption of IL4 was highest when the 300uL volume was used. In vivo, IL4 treatment of ADM was associated with increased vasculogenesis at the implant/host interface at day 21 compared to untreated ADM, resulting in an increase in oxygen saturation within the ADM as seen through repeated oxygen sensing imaging of the window chamber. IL4-treated ADM drove a 31% increase in vascular coverage and a 38% increase in oxygen levels within the ADM at day 21, compared to control samples.

**Conclusion:** The use of ADM as an immunomodulatory drug delivery vehicle has great potential to improve clinical outcomes. Our in vivo model provides the ability to serially assess ADM integration. This novel approach to improve the clinical outcomes of ADM use in implant-based breast reconstruction through modulating the immune response to facilitate integration, provides the foundation for increasing the complexity and designing a clinically useful pharmacologic strategy to gain spatial and temporal control of key signaling cytokine.

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**110 Testing The Effect Of A Novel Hydrogen Sulfide Releasing Peptide On Infected Burn Wounds**

**Afnan Altamimi, MD**, **Yun Qian, PhD**, **Shaina Yates, BS**, **Santu Sarkar, PhD**, **John Matson, PhD**, **Nicole Levi-Polyachenko, PhD**

**1Wake Forest School of Medicine, Winston Salem, NC, USA, 2Virginia Tech, Blacksburg, VA, USA.**

**Purpose:** To evaluate the antimicrobial effects of an H2S releasing dipeptide on *Staphylococcus aureus*.

**Methods:** An H2S releasing peptide hydrogel (S-FE) was synthesized and characterized by standard methodology prior to beginning studies with *S. aureus*. A control peptide that does not release H2S (C-FE) was also developed. The gels were evaluated against two variants of *S. aureus*, Xen29, which is bioluminescent and UAMS-1, which is known to form robust biofilms. Cysteine was used as the trigger for release of the H2S from the S-FE. Thus, the first experiment evaluated the effect of increasing concentrations of cysteine on the growth of *S. aureus*. Secondly, the effect the peptide gels on planktonic *S. aureus* was also developed. The gels were evaluated against two variants of *S. aureus*, Xen29, which is bioluminescent and UAMS-1, which is known to form robust biofilms. Cysteine was used as the trigger for release of the H2S from the S-FE. Thus, the first experiment evaluated the effect of increasing concentrations of cysteine on the growth of *S. aureus*. Secondly, the effect the peptide gels on planktonic *S. aureus* was evaluated with using optical absorption of the culture, complemented by serial dilution and enumeration of the colony forming units (CFUs). The gels were then evaluated against developing or established biofilms to evaluate the potential for the H2S gels to be bacteriocidal or bacteriostatic. Biofilm mass was quantified using crystal violet assay, and bacterial viability was determined by in vivo imaging and CFU enumeration.
An ex vivo porcine skin model was then used to evaluate the potential for the peptide gels to disrupt S. aureus biofilms in burns, and this was then translated into an in vivo porcine burn model to evaluate the safety of the peptides gels and reduction of bacterial burden compared to silver sulfadiazine. The impact of H2S on blood perfusion and wound healing were evaluated using laser Doppler scanning of the burns and histopathological imaging.

Results: Both the S-FE and C-FE peptide hydrogels exhibited reduction in S. aureus in planktonic or biofilm form, indicating an antimicrobial effect of the peptide itself. H2S-releasing S-FE gel which had a better antimicrobial effect in general, suggesting the inhibitory effects of H2S gas. The S-FE and C-FE dipeptide gels suppressed the bacterial ability to grow biofilm. Moreover, the S-FE had a significant antimicrobial effect on an established biofilm compared to C-Fe and a bacterial only group which could indicate the gas ability to infiltrate the biofilm to impact the bacteria directly. The impact of the gels in infected ex vivo porcine skin shows a significant decrease in bacterial burden determined by reductions in both CFUs and photon count emitted from the bioluminescent bacteria. An antimicrobial effect was noticed with the S-FE group compared to the other groups as the infection was cleared before day 14 compared to its control which persisted to day 21. There was no clear acceleration of wound healing with any of the treatments. There were no negative impacts of the dipeptide hydrogels on the health of the animals.

Conclusion: The S-FE and C-FE inhibited bacterial growth, thereby limiting biofilm formation or disrupting established biofilms, and S-FE showed better effects than C-FE. These antimicrobial H2S-releasing dipeptide hydrogels provide a promising new approach to treat wound infections.

Adoptive Transfer Of Tolerogenic Dendritic Cells Promotes Angiogenesis And Wound Healing

Dominic Henn, MD1,2, Clark A. Bonham, Jr.1, Kelln Chen, PhD1, Jagannath Padmanabhan, PhD1, Artem Trotsyuk1, Janos A. Barrera, MD1, Katharina S. Fischer1, Sun Hyung Kwon, PhD1, Michael Januszky, MD, PhD1, Geoffrey C. Gurtner, MD1

Hagey Laboratory for Pediatric and Regenerative Medicine, Division of Plastic and Reconstructive Surgery, Stanford University, Stanford, CA, USA, 2Department of Hand, Plastic and Reconstructive Surgery, Ludwigshafen Trauma Center, Heidelberg University, Ludwigshafen, Germany.

Purpose: Dendritic cells (DCs) are a heterogeneous cell population which critically regulates the adaptive immune response. Depending on their activation status, DCs can also promote peripheral immune tolerance, thus limiting the activation of the immune system and tissue damage. Cell based therapy approaches using DCs have been approved by the FDA and clinical trials using DC immunotherapy are being performed against a variety of cancer types. However, the role of DC therapy for wound healing has not yet been investigated.

Methods: Hematopoetic stem cells (HSCs) were isolated from the bone marrow of green fluorescent protein (GFP) expressing mice and differentiated into DCs over a 7 day in vitro culture period. Tolerogenic DCs (TDCs) were induced by stimulation of cultures with 1,25-dihydroxyvitamin D3 (1α,25(OH)2D3), lipopolysaccharide or dexamethasone and the angiogenic potential of the cells was evaluated by endothelial cell (EC) tube formation assays in transwell as well as direct co-cultures. The protein levels of 52 cytokines were measured in the conditioned media of DC cultures using Luminex multiplex assays. The ability of TDCs to accelerate wound healing was evaluated by treating splinted excisional wounds in C57BL6/J mice weekly with pullulan-collagen hydrogels seeded with 1α,25(OH)2D3-stimulated TDCs, unstimulated DCs or blank hydrogels.

Results: EC tube formation assays showed a significantly higher EC branch number and length when co-cultured with TDCs treated with 1α,25(OH)2D3 both in transwell as well as direct co-cultures. 1α,25(OH)2D3 treatment strongly enhanced vascular endothelial growth factor (VEGF) secretion compared to untreated DCs in vitro (14-fold). Excisional wounds treated with TDC-seeded hydrogels demonstrated significantly faster healing compared to untreated DCs and blank hydrogels.

Conclusion: Our data indicate that the induction of tolerogenicity in DCs by 1α,25(OH)2D3 enhances their secretion of VEGF, thus promoting EC tube formation and accelerating wound healing. Given their ready availability from human blood through established leukapheresis protocols and easy multiplication in