ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Case Report

Novel Hybrid Therapy: Stem Cell Liquid Matrix and Negative Pressure Therapy Promote Sternal Wound Healing

Alice S Ferng[1,4], Alana M Connell[1], Ned Premyodhin[1], Divneet Mandair[1], Tia Pilikian[6], Toshinobu Kazui[1], Ray Runyan[3], John Konhilas[4], Ankit A Desai[2], Samuel Kim[1] and Zain Khalpey[1,3,4,5]*

[1]Department of Surgery, Division of Cardiothoracic Surgery, University of Arizona College of Medicine, United States

[2]Department of Internal Medicine, Division of Cardiology, University of Arizona College of Medicine, United States

[3]Department of Cellular and Molecular Medicine, University of Arizona College of Medicine, United States

[4]Department of Physiological Sciences, University of Arizona College of Medicine, United States

[5]Department of Biomedical Engineering, University of Arizona College of Medicine, United States

[6]Amnio Technology, LLC, Phoenix, United States

***Corresponding Author:** Zain Khalpey, Fellow of the European Board of Thoracic and Cardiovascular Surgeons (FETCS), Department of Surgery, Physiological Sciences, Cellular and Molecular Medicine and Biomedical Engineering, Division of Cardiothoracic Surgery and Cardiology, University of Arizona College of Medicine, 1501 North Campbell Avenue, Tucson AZ 85724, 520.621.0120, Arizona, United States.

Received: April 28, 2016; **Published:** May 12, 2016

Abstract

Since post-surgical sternal wound complications often occur as infections that delay patient healing and discharge, it is important to prevent or treat the wound properly such that complications do not arise. Standard wound care treatments for sternal wound complications include antibiotics, wound debridement, wound VAC or flap coverage for open wounds. However, these treatments may not always promote appropriate healing and infections and additional morbidities may arise. We report the use of injections of a heterogeneous population of amniotic stem cells as an adjuvant therapy to wound VAC procedures to promote quicker post-surgical wound healing. Multiple amniotic stem cell injections were given at the sternal wound site, which resulted in anti-inflammatory and antimicrobial effects that led to faster wound healing and no evidence of infection.

Keywords: *Antimicrobial; Amniotic stem cells; Anti-inflammatory; Wound healing*

Abbreviations: SWI: Superficial Wound Infection; DWI: Deep Wound Infection; PSM: Post Sternotomy Media Stinitis; VAC: Vacuum-Assisted Closure; MSC: Mesenchymal Stem Cell; AmSCs: Amniotic Stem Cells; ECM: Extracellular Matrix; CT: Computed Tomography; WBC: White Blood Cell Count; POD: Post-Operative Day; bFGF: Basic Fibroblast Growth Factor; EGF: Epithelial Growth Factor; TGF: Transforming Growth Factor; PDGF: Platelet Derived Growth Factor

Introduction

Since its introduction in the 1950's, median sternotomy has been the standard of practice for access to intrathoracic organs. Unfortunately, median sternotomy complications such as superficial wound infection (SWI), deep wound infection (DWI) and sternal dehiscence occur in 0.5 to 5% of patients with 0.2 to 3% developing post sternotomy mediastinitis (PSM) [1]. Despite attempts to reduce the incidence of sternotomy wound infections with prophylactic antibiotics, SWI has been reported to affect 3-10% of cardiac surgery patients [2]. Conventional treatments for SWI such as antibiotics, wound debridement, vacuum-assisted closure (VAC) therapy and myocutaneous and/or omental flap coverage often fail consequently, innovative wound therapies including mesenchymal stem cell (MSC)-based treat-

ments have been investigated over the past decade. MSC treatment of acute and chronic wounds have demonstrated accelerated wound closure, angiogenesis and increased epithelialization and granulation tissue formation [3]. Recent focus has been set on amniotic fluid isolated from placental tissue and comprised of multipotent amniotic stem cells (AmSCs), amniotic extracellular matrix (ECM) and multiple growth factors which have shown to promote wound healing and to have antimicrobial effects in animal models [4]. When compared to bone marrow derived mesenchymal stem cells, both populations displayed equivalent immunomodulation properties, but multipotent AmSCs yielded greater stem cell proliferative capacities [4,5]. We report that the anti-inflammatory and antimicrobial properties of amniotic fluid promoted faster wound healing when injected subcutaneously into a dehisced median sternotomy wound.

Case Report

A 79-year-old Caucasian female presents with a past medical history of atrial fibrillation with multiple unsuccessful ablations, sick sinus syndrome, status post pacemaker placement, degenerative mitral valve regurgitation with dyspnea, chronic diastolic heart failure with an EF of 49%, hypertension and spinal reconstruction surgery that resulted in a broken rod. Per patient history, her orthopedic surgeon recommended she have mitral valve repair before attempting to fix the broken rod. The patient underwent elective mitral valve repair with a 26 mm Physio-II annuloplasty ring, primary closure of patent foramen ovale, left atrial appendage ligation and MAZE procedure. Intra operatively, tricuspid valve vegetation (*Enterococcus faecalis*) was appreciated and treated with a six-week course of antibiotics and pacemaker explanation. The patient was discharged home where she underwent cardiac arrest requiring manual chest compressions and an amiodarone bolus while en route to the hospital. She subsequently developed a 1.5 cm and 2 cm separation of her sternotomy incision with thin and fragile skin connecting the two areas of wound dehiscence. A chest computed tomography (CT) showed sternal bony dehiscence. The superior portion of the sternal split was widened with irregular cortical margins and a small collection of gas anterior to the sternal split. Her white blood cell count (WBC) remained within normal limits and blood cultures were negative. The patient was taken to the operating room where the sternal wounds were debrided and washed with vancomycin and bacitracin. Upon inspection, there was no evidence of sternal wires from the surface of the skin and the wound appeared clean and dry without exudate. The sternum was firm and stable to palpation. We injected amniotic fluid containing 2.4 million AmSCs radially along the dermo-epidermal layer and applied a wound VAC. Sternal wound cultures were negative for both aerobic and anaerobic organisms. On post-operative day (POD) 1 following debridement, the patient underwent pacemaker re-implantation. On POD 6 the wound VAC was removed and the wound appeared clean and dry without evidence of infection (Figure 1). After washing with normal saline, we injected another 2.4 million AmSCs into the dermis and closed the wound with sutures and staples. At her one-month follow-up, the patient's stapled incision appeared clean and dry without drainage or erythema around its borders.

Discussion

We present this case of a patient who received adjuvant AmSCs injections for sternal wound and bony dehiscence following external cardiac compressions to demonstrate the potential of amniotic fluid stem cells as a supplemental hybrid therapy for wound management. Although cultures of this patient's wounds did not show evidence of active infection, bacterial contamination of surgical wounds by the patient's skin flora or by bacteria in the local surgical environment is not uncommon. Current literature suggests that 0.5-8.4% of median sternotomy incisions become complicated with infections [6] leading to sternal dehiscence, which is often accompanied by PSM. Moreover, the current case provides some support for use of AmSCs injections to decrease wound healing time. Previous studies have detailed negative pressure therapy for wound infection averaging 27 and 49 days in cases with or without sternal dehiscence, respectively [7]. While active infection was not present in this case, the wound VAC was able to be removed on POD 6.

The advantages of using a wound VAC as a negative pressure dressing for wound management include reduced bacterial load, increased local blood flow to ischemic areas and accelerated granulation tissue formation. A wound VAC can be used as a bridge between wound debridement and sternal reconstruction or as a stand-alone treatment for sternal wound dehiscence. While VAC therapy offers multiple benefits, possible complications include increased risk of bleeding, potential damage to underlying tissue and although very

rare, right ventricular rupture has been reported [8]. Complications from amniotic fluid injections, however, stem from the procedure rather than the fluid: infection at injection site, irritation and possible bruising. We observed that a wound VAC and AmSCs injections are complementary when used as a hybrid therapy without eliciting the negative effects of either treatment. The potential for underlying tissue damage with the use of a wound VAC did not complicate the use of AmSCs injections based on the absence of excessive bruising or erythema on POD1 and POD6.

For our patient, we injected a liquid form of micronized amniotic matrix with amniotic fluid stem cells and amniotic fluid. Determined by an ELISA assay, growth factors were present in the following concentrations in our liquid therapy: basic fibroblast growth factor (bFGF: 40 pg/mL), bone morphogenic protein-2 (4 pg/mL), epithelial growth factor (EGF: 125 pg/mL), transforming growth factor (TGF)-beta 1 (125 pg/mL), platelet-derived growth factor PDGF-AA (10 pg/mL) and PDGF-BB (20 pg/mL) [unpublished data]. Amniotic fluid-derived stem cells share some characteristics with MSCs, but may be even more potent as they are sourced early from the gestational period. AmSCs are an attractive cell source for regenerative medicine due to their high proliferation capacity, multipotency, immunomodulatory activity and lack of significant immunogenicity. AmSCs have the ability to secrete growth factors, immunomodulatory factors (e.g., PTX3-HA), anti-inflammatory molecules (e.g., S100-A8) and can also migrate to sites of tissue injury [9-12]. Anti-inflammatory effects of AmSCs include both direct cell mediated interactions, through inhibiting phyto hemagglutinin-induced lymphocytic activation [12] and indirect release of immunosuppressive factors. In terms of indirect inflammatory control, AmSCs have been linked to the induction of T-regulatory differentiation [13], suppression of peripheral blood mononuclear cell proliferation via Il-10 [14] and release of Il-6 [12]. Secretory profiles of amniotic fluid-derived stem cells have also been reported to contain cytokines relevant for wound healing, such as EGF, FGF, TGF-beta 1 and more [5]. Specifically, the growth factors reported in our therapy are involved in cell regulation, division, migration and proliferation, as well as vascularization. All of these components of amniotic fluid collectively augment wound healing and offer anti-inflammatory and antimicrobial properties [11,12].

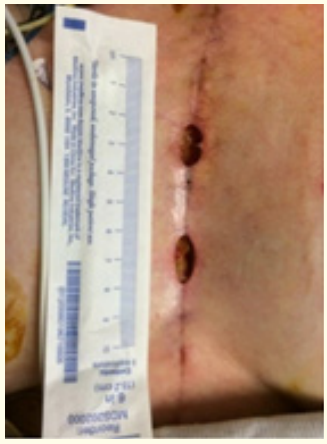

Figure 1: *Wound Appearance on Post-operative Day (POD) #6. Approximately 2.4 million amniotic stem cells were injected following sternotomy as an adjuvant to wound VAC. The wound appeared clean and dry without evidence of infection on POD 6 and wound sizes are approximately 1.5 cm and 2 cm found in the middle of the sternotomy incision. Upper and lower sternal wounds connect with fragile and thin overlying skin. A second 2.4 million stem cell injection was performed at this time and the final wound appeared clean and dry without signs of infection.*

Conclusion

Sternal wound infections following cardiac surgery are an important cause of patient morbidity and mortality. Negative pressure wound therapy with adjunctive administration of a heterogenous mixture of human AmSCs with amniotic fluid containing anti-inflamma-

tory, immunomodulatory and antimicrobial properties, promotes wound healing, reduces hospital costs and stays and minimizes further complications (SWI, DWI, PSM) in patients with sternal wound dehiscence.

Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief.

Acknowledgement

The authors would like to thank the patient and family, University of Arizona College of Medicine, Department of Surgery-Division of Cardiothoracic Surgery.

Conflict of Interests

The author, Zain Khalpey, of this publication has received research support from Amnio Technology, LLC. The terms of this arrangement have been reviewed and appropriately managed by the University of Arizona in accordance with its conflict of interest policies. Furthermore, author, Tia Pilikian, is a paid employee of Amnio Technology, LLC.

Bibliography

1. Olbrecht VA., *et al*. "Clinical Outcomes of Noninfectious Sternal Dehiscence after Median Sternotomy". *Annals of Thoracic Surgery* 82.3 (2006): 902-907.

2. Chikwe J., *et al*. "Cardiothoracic Surgery". Oxford: *Oxford University Press* (2006).

3. Maxson S., *et al*. "Concise Review: Role of Mesenchymal Stem Cells in Wound Repair". *Stem Cells Translational Medicine* 1.2 (2012): 142-149.

4. Yang JD., *et al*. "Effect of Amniotic Fluid Stem Cells and Amniotic Fluid Cells on the Wound Healing Process in a White Rat Model". *Archives of Plastic Surgery* 40.5 (2013): 496-504.

5. Yoon BS., *et al*. "Secretory Profiles and Wound Healing Effects of Human Amniotic Fluid-Derived Mesenchymal Stem Cell". *Stem Cells and Development* 19.6 (2010): 887-902.

6. Song DH., *et al*. "Vacuum Assisted Closure for the Treatment of Sternal Wounds: The Bridge between Debridement and Definitive Closure". *Plastic and Reconstructive Surgery* 111.1 (2003): 92-97.

7. Tang AT., *et al*. "Novel Application of Vacuum Assisted Closure Technique to the Treatment of Sternotomy Wound Infection". *European Journal of Cardio-Thoracic Surgery* 17.4 (2000): 482-484.

8. Dickie SR., *et al*. "Definitive Closure of the Infected Median Sternotomy Wound: A Treatment Algorithm Utilizing Vacuum-Assisted Closure Followed by Rigid Plate Fixation". *Annals of Plastic Surgery Journal* 56.6 (2006): 680-685.

9. Cruciani L., *et al*. "Pentraxin 3 in Amniotic Fluid: A Novel Association with Intra-amniotic Infection and Inflammation". *Journal of Perinatal Medicine* 38.2 (2010): 161-171.

10. Hsu K., *et al*. "Anti-Infective Protective Properties of S100 Calgranulins". *Anti-Inflammatory and Anti-Allergy Agents in Medicinal Chemistry* 8.4 (2009): 290-305.

11. De Coppi P., *et al*. "Isolation of Amniotic stem Cell Lines with Potential for Therapy". *Nature Biotechnology* 25.1 (2007): 100-116.

12. Moorefield EC., *et al*. "Cloned, CD117 Selected Human Amniotic Fluid Stem Cells are Capable of Modulating the Immune Response". *PLOS One* 6.10 (2011): e26535.

13. Pianta S., *et al*. "Amniotic Membrane Mesenchymal Cells-Derived Factors Skew T cell Polarization toward Treg and Downregulate Th1 and Th17 Cells Subsets". *Stem Cell Reviews and Reports* 11.3 (2014): 1-14.

14. Luo C., *et al*. "Human Amniotic Fluid Stem Cells Suppress PBMC Proliferation through IDO and IL-10-Dependent Pathways". *Current Stem Cell Research and Therapy* 9.1 (2014): 36-45.

Volume 3 Issue 3 May 2016
© All rights reserved by Zain Khalpey., *et al.*