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Contractor/Company Name: 81 MSGS/SGCQ

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Contract Number: N/A

Title of the Report: Omental Lipid-Coated Mesh

Date of the Report: 16 June 2011

Principal Investigator: Capt. Andrew Hall, 228-376-4901, Fax: 228-376-0128, andrew.hall.2@us.af.mil

Sponsoring Organization:

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Pages: 6

Words: 1479

Tables: 1

Photos: 1

References: 8

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Omental Lipid-Coated Mesh

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Acknowledgements:

The opinions and/or assertions contained herein are solely those of the authors and should not be construed as reflecting those of the US Air Force, Department of Defense, or government.

ABSTRACT

OBJECTIVE: Our objective was to determine the possible potential for improved incorporation of omental-coated biologic mesh.

MATERIALS AND METHODS: Twenty mice had omental-coated mesh placed on one flank and noncoated control mesh placed on the other. Mice were harvested at one week post implantation, and the results determined by histiologic examination.

RESULTS: There was no objective statistical difference in the twenty mice in terms of acute (p=0.232), chronic (0.165), or neoangiogenesis (High- p=0.436, Low- p=0.264).

CONCLUSION: While no statistically significant results were found with this study, the identification of heavier acute inflammation and broader vascular proliferation with omental coating is encouraging.

INTRODUCTION:

Angiogenesis is one of the principle functions of the mammalian omentum. Prior research has indicated that the ability to initiate blood vessel formation may be related to the lipids contained within the omentum.^{1,2} Potential medical applications of a locally angiogenic substance, especially one as cheap and plentiful as mammalian omentum, are numerous. This paper investigates one potential medical application - improvement of mesh incorporation.

MATERIALS AND METHODS:

Omental Lipid Fraction Preparation:

The omental lipid fraction was prepared based on methods described in prior literature with some modification. Eighty-five grams of omentum from multiple pigs (*Sus Scrofa domestica*) was homogenized in a 2:1 chloroform to methanol mixture. Following homogenization, the mixture was centrifuged for 20 minutes with supernatant removed. The supernatant was then purified via use of a rotary evaporator. The purified lipid extract was again centrifuged with the white lipid layer extracted for use. Lipid between use was stored at -70° C. In prior studies, phosphate-buffered saline was included in the purification process. This was eliminated because the saline and lipids are immiscible, and the water, naturally present in the tissue, served as an ample bulking agent for ease of purification processes.

Biologic Mesh Preparation and Implantation:

Biologic mesh (Permacol, Tissue Science Laboratories, Inc.) was prepared by placing the mesh in preprepared omental lipid reheated to 50° C. After preparing the mesh, twenty mice were placed under general anesthesia using isoflorane via gassing down an induction chamber or by face mask. Mice were then prepped sterile fashion. A 1x1 cm piece of omental-covered biologic mesh was placed subcutaneously on the left flank adjacent to the muscular fascia. The mesh was secured with two vicryl sutures. A control group 1x1 cm piece of plain mesh was placed in an identical fashion on the right flank.

On post-operative day seven, mice were euthanized and the abdominal wall with mesh harvested. The combination of mesh and abdominal wall was placed in formalin and prepared for microscopic examination to evaluate evidence of angiogenesis and ingrowth.

Gross Examination/Macroscopy:

The mouse flanks were received fresh on the day of harvesting, one right and one left, from each of the 20 mice. They were labeled and pinned out in 10% formalin solution. They were left in fixative for 36-72 hours, then grossly examined, measured, and serially sectioned, with all tissue then paraffin embedded and cut into 5 μ m thick sections. Standard hematoxylin and eosin (H&E)-stained slides were created for microscopic examination.

Microscopic examination:

The criteria for examination were adapted from Zheng et al.³ The three variables examined were acute inflammation, chronic inflammation, and neovascularization. These three variables were measured by light microscopic identification on H&E-stained slides. Three separate high-power fields (HPF, 400x total

magnification) were selected from the area directly adjacent to the surgically implanted graft material, and for each of the three variables a score was given per HPF. Areas with greater inflammation and vascularization were selected for examination in preference over areas with little inflammation or vascularization. Two separate pathologists (NL and CG) reviewed each slide and reconciled differences in scoring opinion. Neovascularization was considered any group of small-caliber capillaries which were situated adjacent to the graft material or within the loose connective tissue surrounding the graft material. It was graded on a scale of absent (0), mild (1), moderate (2) and marked (3). Not counted were large-caliber vessels or those that were in the dermis or dense fibrous connective tissues. Likewise, acute and chronic inflammation was only counted when it existed adjacent to the graft material, or if it was situated in the loose connective tissue immediately surrounding the graft material. All inflammatory cells within the dermis and dense connective tissue or those that were part of an abscess were omitted. The inflammatory response was also graded on a scale of absent (0), mild (1), moderate (2), and marked (3). As an additional internal control, three quiescent HPFs were selected on each side and scored for vascularity (Low - Table 1). The scores for each variable and control were recorded and the averages were calculated. The average values for acute inflammation, chronic inflammation, and neovascularization were recorded for comparison between mice and between left (control) and right (omental-coated mesh) flanks. Statistical analysis was done using a nonparametric test based on ranks with the statistical package R.^{4,5}

RESULTS:

Microscopic analysis revealed unstatistical differences between the control and coated mesh in all measures (Table 1). While there was higher acute inflammation in the coated side and, when present on the coated side, very pronounced neoangiogenesis compared to the control (Figure 1), it did not translate into increased neoangiogenesis at the one week interval across all mice.

Measurement	Control (Left)	Omental-Coated Mesh (Right)	
Acute Inflammation	0.8	1.2	p=0.232
Chronic Inflammation	1.1	0.9	P=0.165
Vacularity (High)	1.3	1.1	P=0.436
Vascularity (Low)	0.1	0.2	P=0.624

Figure 1: Comparison of histiologic results. a) Left side with omental lipid; b) right side without. Areas of neoangiogenesis marked with 'x'.



DISCUSSION:

Neovascularization is a well-studied phenomenon, and its physiological benefits are well known. It is a vital ingredient in resistance to infection and wound healing. These benefits would be important in improving surgical care for all wounds, but they may greatly improve outcomes in abdominal wall hernia repair. If cheap and effective promotion of neovascularization could be initiated, we might be able to improve upon current techniques and materials for hernia repair.

The exact mediator of omental-lipid angiogenesis has not been identified precisely. In experimentation done on multiple species of mammalian omental lipids, theorized active factors include mono- and diglycosylceramides, globoseries glycolipids, and gangliosides.⁶ We do know that there have been proven responses with their use in multiple injury models including ischemic wounds and orthopedic injuries.^{1,7} If omental lipids can benefit orthopedic and ischemic wounds, then the subsequent inflammatory and neoangiogenic response may also benefit the healing of hernia defects repaired with mesh.

Pairing this angiogenic and inflammatory ability with biologic mesh is the goal. The use of biologic mesh is now becoming standard in repairs of abdominal defects with contamination, or when synthetic mesh is contraindicated. However, these meshes are plagued with high hernia recurrence rates. If one can stimulate angiogenesis and growth factors earlier in the mesh, this may lead to quicker fascial ingrowth and incorporation into native fascia, quicker recovery, quicker time to final strength, and ultimately less recurrence and resistance to infection.

Though this small study did not demonstrate significant increases in neovascularization, we think that this principle has promise in the area of hernia repair and mesh preparation. Improvements include increasing time until harvest to allow for inflammatory and neoangiogenic mediators to proliferate, i.e. macrophages. As seen in the study, acute inflammation was prominent. Having higher acute inflammation may mean a higher number of 'chronic' inflammatory cells later, but a one-week harvest was likely too early to tell. Another improvement would be a more porous biologic mesh. Permacol is porcine dermis prepared with crosslinking to increase durability. This crosslinking naturally resists degradation and incorporation by the body and would not ordinarily show much ingrowth in one week's time. Porosity would also allow for more penetration of the large lipid molecules and room for vascular proliferation.⁸

As mentioned, the objective results are not conclusive, and further research is needed. It appeared, though, when observing the results histiologically, when the omental coating did work, it worked very well (Figure 1). This was not seen across all mice, but some evidence can be seen with a higher amount

of vascularity in the lowest vascularity areas. There tended to be a broad and more uniform response on the omental mesh side when compared to the isolated foci of neoangiogenesis on the control side. The spotty nature of control-side angiogenesis may be a result of migration of omental fats from the experimental side to the control side through the very loose subcutaneous tissue of the mouse.

Future studies will be directed at noncrosslinked mesh implanted for longer duration as well as synthetic mesh. Also, future studies will be directed at the clinical benefits of mesh preparation with omental lipids, such as in vivo tensile strength, recurrence rate, and infection. If benefit is proven, this method will be a cost-effective way to prepare biologic and possibly synthetic meshes for use in hernia repair. This would potentially save healthcare dollars with quicker recovery, less infection, and lower recurrence rates.

CONCLUSIONS:

The possibilities for omental lipids cost-effective and widespread use are tantalizing. Compared to the cost of a known neovascular growth factor such as VEGF at approximately \$2700 for 1 mg, the large-scale supply of the American agricultural sector could promise almost limitless quantities of omentum at an affordable price. While no statistically significant results were found with this study, the identification of heavier acute inflammation and broader vascular proliferation with omental coating is encouraging and will lead to refinement of the process and a useable product for both military and civilian medicine.

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