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TITLE: Investigation of Angiogenic Mechanisms Involved in Omentum Transplantation

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ABSTRACT

Investigation of Angiogenic Mechanisms Involved in Omentum Transplantation

The omentum is an intra-abdominal fat pad. It is a well-vascularized structure that is sometimes referred to as the "policeman of the abdomen" for its proclivity to adhere to sites of intra-abdominal inflammation or injury. It is also recognized for the ability to provide effective tissue reinforcement and bring a source of vascular supply to areas of inflammation and healing. In clinical practice, the omentum is often used to buttress surgical repair – and to ensure adequate vascularization of healing tissues. A limitation in its use is that many times the mobility of the omentum is constrained because it is time-consuming and not always feasible. Thus, this project addressed the question of whether the omentum can be used as a free, autonomous graft by (1) determining that a free omental graft remains viable, and (2) characterizing the neo-vascularization of the recipient bed.

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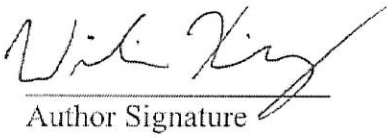

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Abstract:

The omentum is an intra-abdominal fat pad. It is a well-vascularized structure that is sometimes referred to as the "policeman of the abdomen[1]" for its proclivity to adhere to sites of intra-abdominal inflammation or injury. It is also recognized for the ability to provide effective tissue reinforcement and bring a source of vascular supply to areas of inflammation and healing. In clinical practice, the omentum is often used to buttress surgical repair – and to ensure adequate vascularization of healing tissues. A limitation in its use is that many times the mobility of the omentum is limited by physical constraints. This project examined the hypothesis that the omentum can be used as a free, autonomous graft by (1) determining if a free omental graft remains viable, and (2) characterizing the neo-vascularization of the recipient bed.

Introduction:

In humans, the greater omentum is a fatty apron located in the abdominal cavity that covers the stomach and most abdominal organs, whereas in mice, it exists as a "stripe" of fatty tissue that runs longitudinally along the stomach.[1] Initially, the omentum was believed to be an inert structure. However, 20th century discoveries of the omentum's capacity to adhere to and seal microperforations of the human bowel led the British surgeon Rutheford Morison to bestow the moniker "policeman of the abdomen" upon the omentum, in reference to its newfound capabilities.[2] Today, the omentum is recognized as a highly vascular endocrine organ, and its versatility is known to extend to the secretion of neurotransmitters, inflammatory mediators, and growth factors.[3-6]

Amongst the omentum's many properties is the capacity to stimulate angiogenesis in neighboring tissue, indicating that it is an effective promoter of blood vessel growth.[7] Neovascularization is valuable because it accelerates wound healing by increasing the production of collagen for the formation of new granulation tissue.[8] Unsurprisingly, the omentum is useful in clinical practice, and repairs of enteric, bladder and vaginal fistula are commonly accompanied by the application of the omentum in this manner.[9] However,

the omentum will not always reach a surgical site because the site of surgery can be deep in the pelvis and/or the mobility of the omentum is limited. In these situations, a segment of omentum may be partially separated from the main body so that it can be applied to the surgical site. There has been far less research into the efficacy of using a completely detached, "free" graft, however. One of the few experiments conducted to evaluate such a non-vascularized graft was done in canines and found that while thick omentum grafts were nonviable, thin grafts could be successfully used, supporting similar application in humans.[10] Despite this finding, the limited body of knowledge regarding the use of the omentum as a free autonomous graft means that is not exactly known how the omentum is able to survive after losing its initial intact blood supply.

To address this question, this study first evaluated the ability of omental tissue to survive by scavenging a different blood supply following transplantation to a new location. Following this, the surrounding vasculature was analyzed to propose a potential means for graft survival.

Methods:

This study was performed using autonomous (free) grafts in wild-type C57B6/J mice. In each of 2 donor mice, the entire stripe of omentum (Fig. 1) was removed and smeared with the intravital fluorescent powder dye Fast Blue (FB).

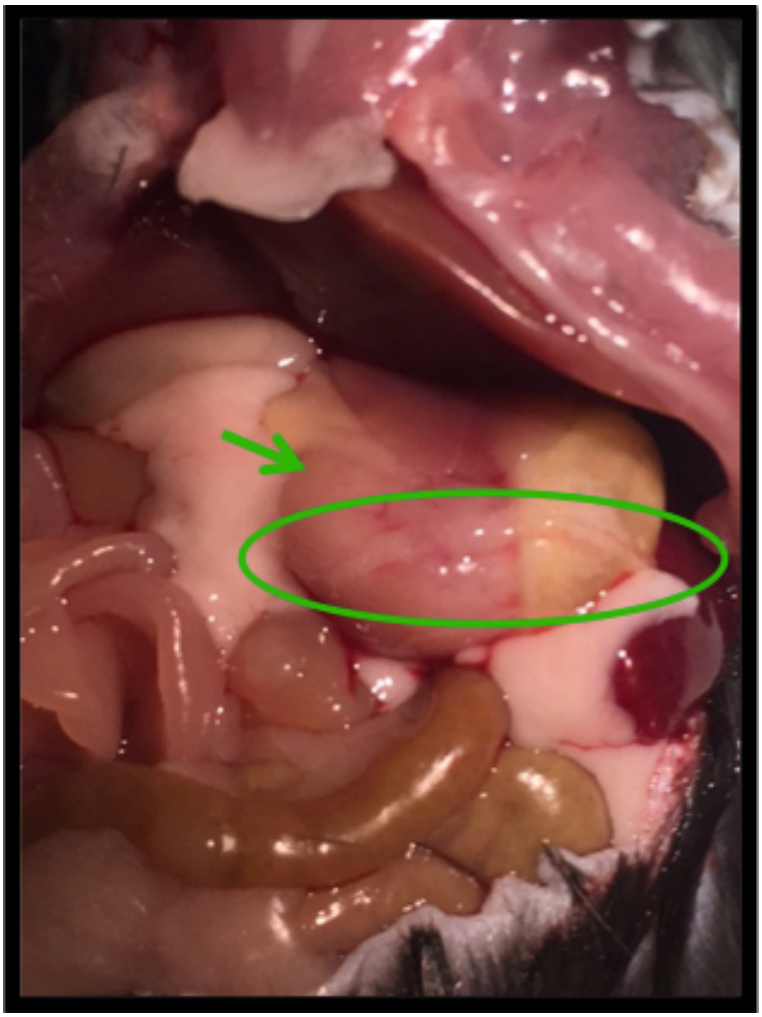

Figure 1: Stripe of omentum in the mouse running across the stomach (denoted by green arrow). The omentum (circled in green) was isolated and excised in donor WT C57B6/J mice. This tissue was then smeared with powdered Fast Blue (FB) dye and transplanted to the bladders of the host mice.

Each labeled graft of omentum was then transplanted onto the bladder of a host mouse (n=2). These host mice were sacrificed 7 (n=1) and 13 (n=1) days after surgery. The sole omentum graft was removed from the 7-day post-op host, while in the 13-day post-op host, the graft was marked with black Davidson Marking System® dye and removed *en bloc* along with its adjoining abdominal organs. After fixation, all tissue was embedded in paraffin and sectioned (5μm). Fluorescence imaging to detect FB was

performed using a Nikon A1R confocal microscope. Hematoxylin and Eosin (H&E) staining was done on serial sections to visualize cell structure, including nuclei.

Results:

The presence of blue fluorescence was detected in the omentum grafts collected both 7 and 13 days after surgery. Histologic evaluation of the omentum taken after 7 days (Fig. 2a), using a corresponding H&E image to the section of tissue exhibiting fluorescence, revealed the presence of intact nuclei in the adipocytes.

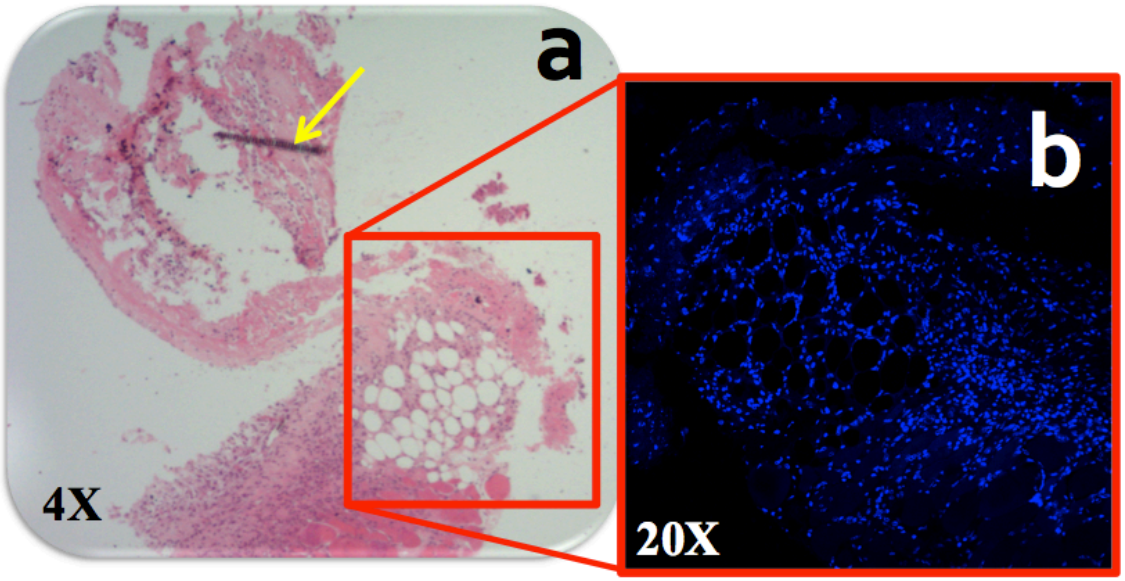

Figure 2 (a,b). Omentum graft 7 days following transplant in female C57B6/J wild-type mouse. (a) Hematoxylin & Eosin (H&E) image of omentum. Arrow denotes suture used to secure graft. (b) Corresponding section of surviving Fast Blue-labeled omentum as depicted by fluorescence microscopy.

These findings were corroborated by those for the omentum taken 13 days after transplantation (Fig. 3a). Similarly, the corresponding area of the omentum graft exhibiting blue fluorescence was shown to contain adipocytes with intact nuclei after H&E staining. However, the presence of necrotic tissue, as evidenced by local cell debris

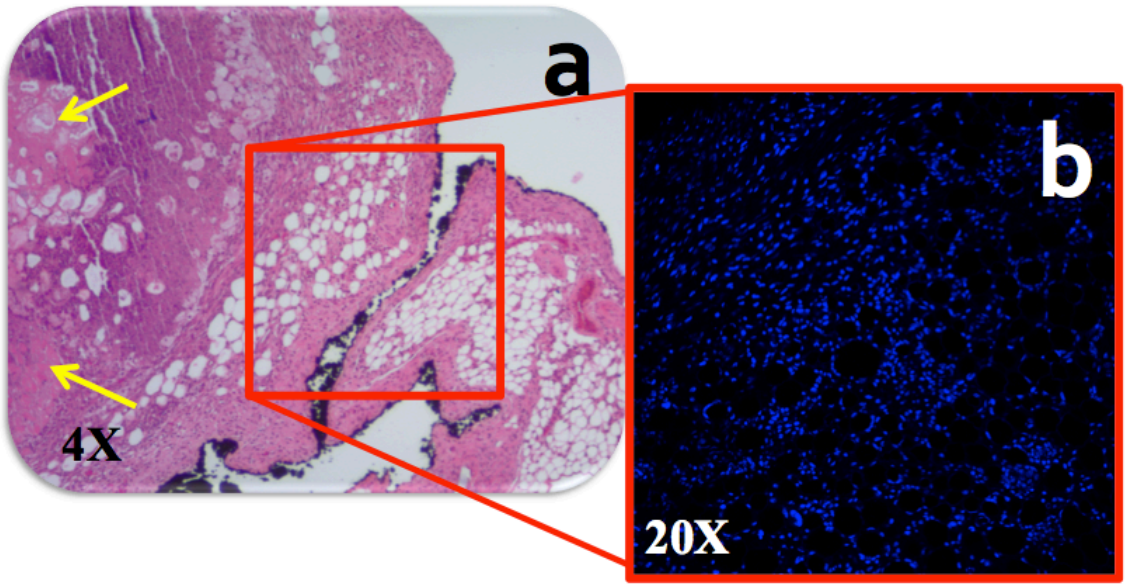

Figure 3 (a,b). Omentum graft 13 days following transplant in male C57B6/J wild-type mouse. (a) H&E image of omentum. Arrows denote partial area of necrotic tissue. (b) Corresponding section of surviving Fast Blue-labeled omentum as depicted by fluorescence microscopy.

and nuclear disintegration, was also found in the outskirts of this longer-standing graft, away from the transplant site at the bladder.

Subsequent histologic inspection of the host bladder revealed the presence of a blood vessel containing red blood cells within the detrusor muscle wall (Fig. 4). Earlier serial sections showed a blood vessel extending from the omentum graft tissue towards the bladder, suggesting that it formed following surgery. (Fig. 5).

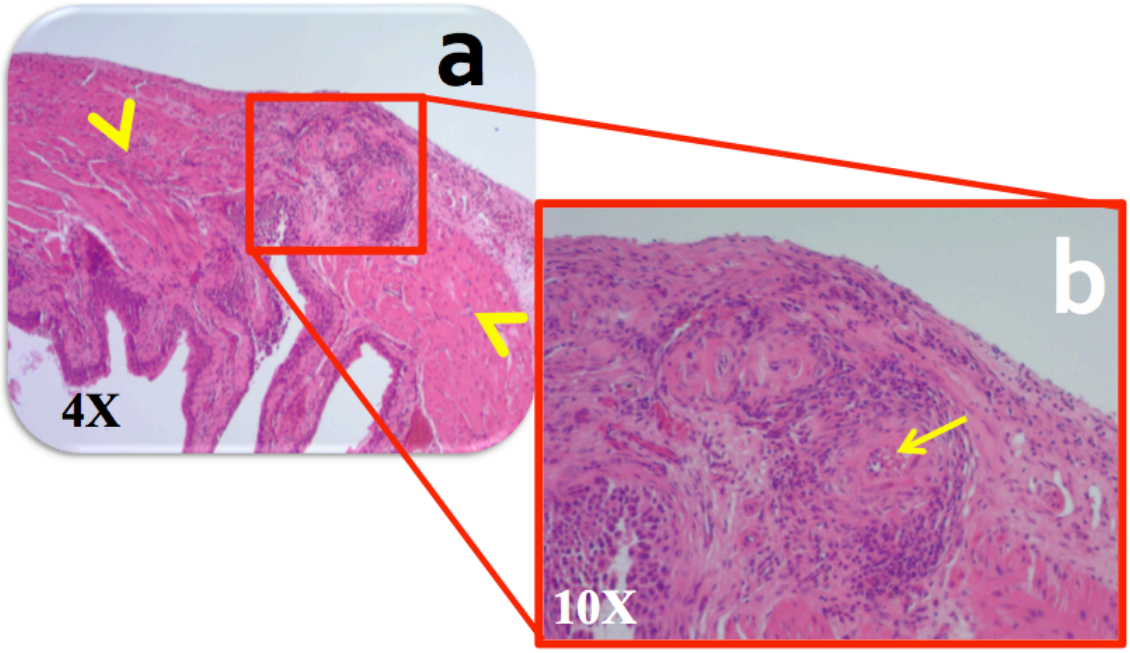

Figure 4 (a,b). Omentum graft and host bladder 13 days following transplant in male C57B6/J wild-type mouse. (a) H&E image depicting interruption of detrusor muscle of bladder (arrow heads) by a blood vessel (boxed in red). (b) Enlarged H&E image of the blood vessel. Arrow denotes red blood cells within blood vessel.

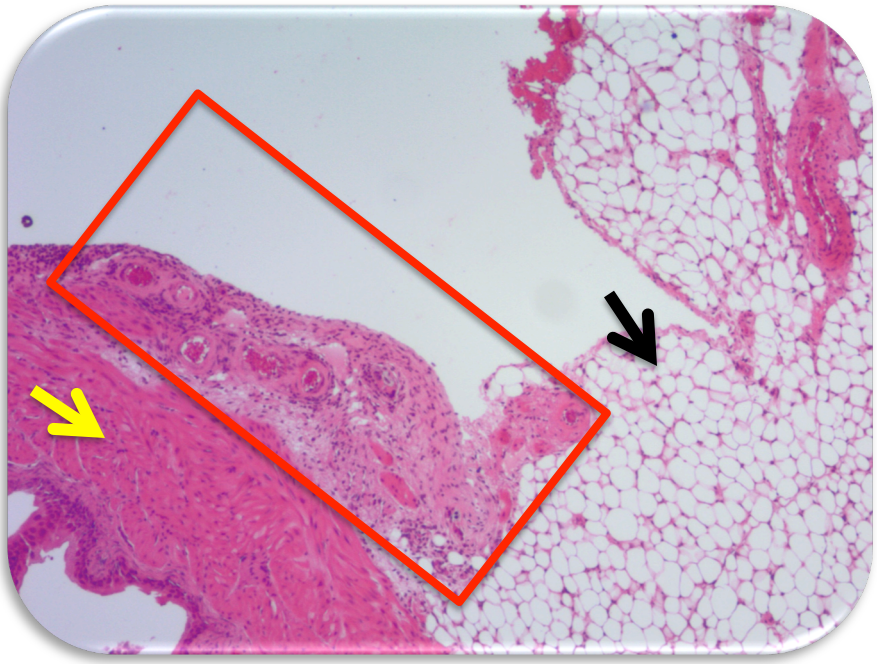

Figure 5: H&E image of Omentum graft and host bladder 13 days following transplant in male C57B6/J wild-type mouse. Blood vessel boxed in red is seen to be extending between the fatty omentum graft (black arrow) and the detrusor muscle of the bladder (yellow arrow).

Discussion:

The presence of fluorescent Fast Blue-labeled tissue, combined with histologic confirmation of normal omentum morphology and healthy adipocytes in this region, indicates that at least part of the omentum graft remained intact both 7 and 13 days after surgery. Moreover, the traceable progression of a large blood vessel from the omentum graft towards and eventually into the detrusor muscle wall of the bladder (Fig. 4 a,b) likely means that this vessel formed after the initial surgery. Together, these findings suggest that the free, autonomous omentum graft remains viable and is able to induce vascularization from adjacent tissues. It also suggests – unexpectedly – that the devascularized state of the free graft exerts a very powerful angiogenic effect on adjacent structures.

That being said, the discovery of necrotic tissue near the omentum graft taken 13 days after surgery suggests that complete survivability of transplanted omentum is not supported by these studies. However, the observation that the necrosis occurs distally to the transplant location (bladder surface) seems to coincide with the aforementioned canine study that showed viability of thin free grafts, but not thick ones.[10] Thus, it could tentatively be hypothesized from the combination of these findings that angiogenesis stimulated by the free omentum is insufficient to revascularize the entirety of the graft. Before further evaluating this idea, though, it is first necessary to confirm that the necrotic tissue was indeed formerly part of the original omentum graft. Because the transplantation process is quite invasive, as significant rearrangement of the abdominal organs and fat is required to gain access to the bladder, it is plausible that host tissue adjacent to the graft was traumatized during surgery. Unfortunately, the current studies

would not have been able to distinguish between this event and cell death in the actual graft itself, so further conclusions stemming from the belief that part of the omentum graft failed would be limited to a speculative nature at best.

The described studies here show for the first time that the omentum will achieve at least partial survivability following transplantation in mice. This finding has wide-reaching ramifications, as it establishes that omentum studies are feasible in the mouse model, which is one of the most versatile model systems for experimentation. In particular, the availability of genetically engineered transgenic mice opens up several avenues that could be pursued from this point on. Future experiments would ideally repeat the omentum transplants with Red Fluorescent Protein (RFP) and Green Fluorescent Protein (GFP) transgenic mice as the omentum donors and hosts. This would allow newly developed blood vessels to be easily determined as originating from the omentum graft, the recipient bed, or both, which will greatly elucidate the neovascularization process that occurs following omentum transplantation.

With a clearer understanding of how the omentum operates mechanistically, experiments can be designed with a focus on expanding the realm of possibilities for the use of omental grafts in the clinic. Genetically equivalent inbred mice, as demonstrated here, are important for facilitating these studies because they make it possible to transplant the omentum from one organism to another without eliciting a harmful immune response. In this way, differences in efficacy could potentially be studied between omentum grafts containing various genetic disorders. For example, atherosclerosis is a condition in which blood vessels thicken and limit circulation throughout the body, putting affected patients at risk for poor recovery from surgical

procedures. Mouse models for atherosclerosis, such as apoE -/-, could be used to determine whether omentum grafts with this knockout gene are less effective at inducing angiogenesis than those with normal genotypes.[11] Depending on what these studies find, omentum graft transplants could eventually become a regular clinical practice, as it is for other organs.

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