A Preclinical Biceps Femoris Musculocutaneous Flap for Studying Ischemic Conditions

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INTRODUCTION: Ischemia-reperfusion (I/R) injury is a pathology that affects transplanted tissues. Currently, there is no treatment for reperfusion injury. There are limited composite tissue models. A single tissue evaluation is not clinically translatable. Akyurek1 first described the dissection of a rat biceps femoris musculocutaneous flap which was islanded along the caudal-femoral-popliteal axis and sutured in situ or transposed to a sacral defect. The arterial supply of the biceps femoris musculocutaneous flap has been described. 1,3 No study has employed the pedicle-clamping technique to develop an I/R model using the biceps femoris musculocutaneous flap. The purpose of this study is to utilize the biceps femoris musculocutaneous flap as a composite tissue I/R model. We hypothesize that selective ligation of peripheral collaterals with the addition of the pedicle clamping technique will result in greater injury to the biceps femoris musculocutaneous flap compared to flap elevation alone.

METHODS: IACUC approval was obtained. This study utilized an in situ (nerve-sparing) preclinical biceps femoris musculocutaneous flap in a rat with or without the addition of I/R. A total of 7 Sprague Dawley rats (2 females and 5 males) weighing 290 to 580g were used for this pilot. The rats were randomized into three groups: 1) I-2hrs/R (n=2), 2) I-3hrs/R (n=3), or 3) sham (n=2). Sham animals had circumferential flap elevation alone without I/R. I/R was defined as selective ligation of collaterals with the addition of pedicle clamping. A 4cmx2cm elliptical skin incision was made along the posterior thigh over the biceps femoris. The biceps femoris was then circumferentially elevated off its tendinous attachments in all animals. The I/R groups underwent microsurgical dissection where the flap was islanded along the caudal-femoral popliteal axis via selective ligation of collaterals. 1,3 Next, 6-0 silk sutures were used to temporarily occlude the popliteal vessels (Fig 1A). Ischemia was induced by placing atraumatic clamps across the popliteal and pedicle for either 2 or 3hrs. Clamp release signaled reperfusion. The flap was sutured in situ. Laser speckle imaging was used to characterize perfusion changes before, during ischemia or reperfusion, and during the postoperative period until euthanasia (POD1 or POD3). The perfusion was quantified as a flap blood flow ratio, a ratio that compares the specific-perfusion pressure unit to the operative limb’s baseline value prior to surgery. The temperature ratio of the skin-paddle was also evaluated and similarly compared to the operative limb’s baseline temperature prior to surgery. At POD3, the histology of the I/R, normal, and sham tissues (n=1 per group) were compared.

RESULTS: I/R groups had edematous and pale muscles, and the skin-paddle was thickened/erythematous compared to the contralateral normal side(Fig 1B). Circumferential elevation of the biceps femoris musculocutaneous flap increased the blood-flow blood ratio. Compared to baseline, the I-2hr or I-3hr groups had visibly reduced flap-blood flow ratio during ischemia (0.46 & 0.70, respectively) and increased flap-blood flow ratio during the reperfusion period (1.21 & 1.18, respectively), although this trend did not reach statistical significance. Compared to baseline and circumferential flap elevation, two-way ANOVA revealed a significant reduction in the skin-paddle’s temperature ratio during the ischemic period in the I-2hr (0.81) and I-3hr (0.82) groups (Fig 1C-D, p<0.01). Sham and I/R groups had histological evidence of skin and muscle injury, although a greater percentage of myonecrosis was seen in the I/R group (50% vs. 25% in sham).

DISCUSSION: Our preliminary data characterizes the dynamic perfusion and temperature changes that occur to a composite tissue such as the biceps femoris musculocutaneous flap in the presence of I/R. The addition of the pedicle clamping technique and then later release to simulate I/R has the potential to induce more histological evidence of muscle injury than circumferential flap elevation alone. The skin appears more resilient, however, this requires further validation given our sample size. The future direction of our study involves increasing the number of animals and evaluating injury at various times. Ultimately, we hope to confirm that our I/R model can induce reperfusion injury to a composite tissue through the presence of pathology-specific biomarkers.

SIGNIFICANCE/CLINICAL RELEVANCE: The annual need for composite tissue reconstruction is reportedly 7 million people. 4 Composite tissues transplanted during vascular tissue allografts and free-flap procedures can be variably affected by reperfusion injury. The significance of our work includes the investigation of reperfusion injury in both skeletal muscle skin which allows us to identify specific patterns associated with each of these tissues. This could then be used for targeted therapy to either potentially treat or prevent reperfusion injury of the skin or skeletal muscle. We would also establish a clinically relevant composite tissue I/R model that can be extrapolated to study the effects of prolonged ischemia after VCA, free-flap reconstruction, and/or limb trauma. If our hypothesis is proven, our model can provide future researchers with a prototype suitable for studying transplantation related to the musculoskeletal system which would also permit the evaluation of neuromotor recovery which is not feasible with the commonly used rodent skin flap model.

REFERENCES:

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Figure 1: Ischemia-Reperfusion (I/R) model utilizing the bilateral femoral myocutaneous flap (BFMCF).

A) **I/R Surgical technique**: Elliptical skin incision is made and the perforator to the skin-paddle is identified and protected. The BFMCF is circumferentially excised from its attachments. Peripheral collaterals to the BFMCF are ligated. 6-0 sutures are used to obtain proximal distal control of the popliteal vessels. Clamps (blue=popliteal vein, green=popliteal artery) are applied to the popliteal vessels and the pelvis for 2 or 3 hours (Ischemia). Clamps are removed to reperfuse the BFMCF. The BFMCF is closed in layers. Congestion is seen in I/R groups. I/R smacked in sham.

B) **Gross Morphology**: Edema, lighter muscle and epidermal thickening/thrombi/mesothelial growth to the skin in I/R versus normal.

C) **Laser speckle imaging (LSI)**: I/R had visibly lower flap blood flow (FBB) during ischemia and higher FBB during reperfusion.

D) **FB ratio (FBB) & Temperature ratios (TR) to baseline (BSL)**

E) **Histology**: Sham and I/R had injuries; both had dermal infiltrates (circle). However, I/R had more severe myonecrosis (50%) vs sham (25%). None seen in normal.