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PRE-CLINICAL RESEARCH

Extracellular Matrix Hydrogel Promotes Tissue Remodeling, Arteriogenesis, and Perfusion in a Rat Hindlimb Ischemia Model

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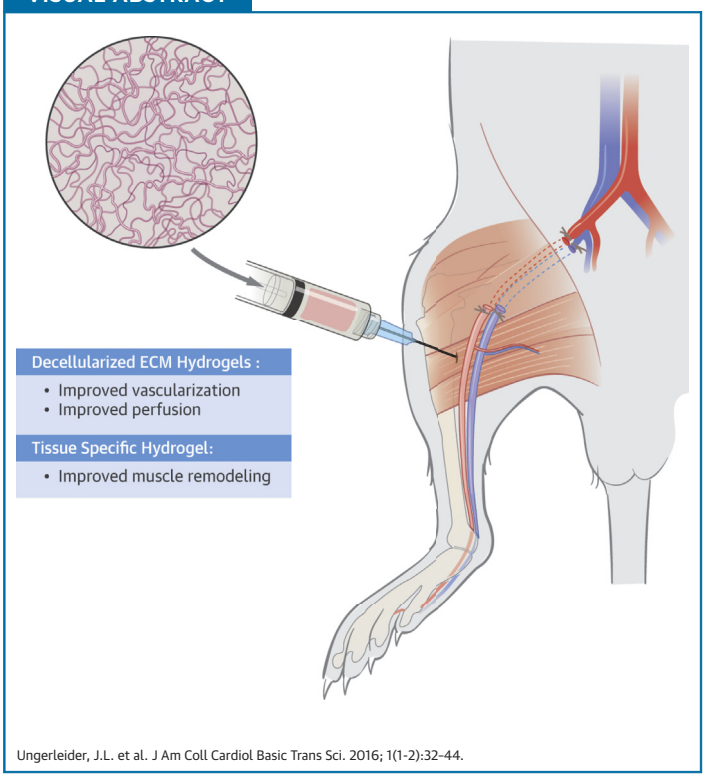
VISUAL ABSTRACT



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HIGHLIGHTS

- **Although surgical and endovascular revascularization can be performed in patients with peripheral arterial disease (PAD), 40% of patients with critical limb ischemia do not have a revascularization option.**
- **The efficacy of an injectable tissue-specific skeletal muscle extracellular matrix (ECM) hydrogel and a human umbilical cord-derived ECM hydrogel were examined in a rodent hindlimb ischemia model.**
- **Although both biomaterials increased tissue perfusion 35 days post-injection, likely through arteriogenesis, the skeletal muscle ECM hydrogel more closely matched healthy tissue morphology.**
- **Transcriptomic analysis indicates the skeletal muscle ECM hydrogel shifted the inflammatory response, decreased necrosis/apoptosis, and increased blood vessel and muscle development.**

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SUMMARY

Although surgical and endovascular revascularization can be performed in peripheral arterial disease (PAD), 40% of patients with critical limb ischemia do not have a revascularization option. This study examines the efficacy and mechanisms of action of acellular extracellular matrix-based hydrogels as a potential novel therapy for treating PAD. We tested the efficacy of using a tissue-specific injectable hydrogel derived from decellularized porcine skeletal muscle (SKM) and compared this to a new human umbilical cord-derived matrix (hUC) hydrogel, which could have greater potential for tissue regeneration because of the younger age of the tissue source. In a rodent hindlimb ischemia model, both hydrogels were injected 1-week post-surgery and perfusion was regularly monitored with laser speckle contrast analysis to 35 days post-injection. There were significant improvements in hindlimb tissue perfusion and perfusion kinetics with both biomaterials. Histologic analysis indicated that the injected hydrogels were biocompatible, and resulted in arteriogenesis, rather than angiogenesis, as well as improved recruitment of skeletal muscle progenitors. Skeletal muscle fiber morphology analysis indicated that the muscle treated with the tissue-specific SKM hydrogel more closely matched healthy tissue morphology. Whole transcriptome analysis indicated that the SKM hydrogel caused a shift in the inflammatory response, decreased cell death, and increased blood vessel and muscle development. These results show the efficacy of an injectable ECM hydrogel alone as a potential therapy for treating patients with PAD. Our results indicate that the SKM hydrogel improved functional outcomes through stimulation of arteriogenesis and muscle progenitor cell recruitment. (J Am Coll Cardiol Basic Trans Sci 2016;1:32–44)

More than 27 million patients in North America and Europe have been diagnosed with peripheral artery disease (PAD) (1). This disease leads to an estimated 120,000 patients in the United States and 100,000 patients in Europe requiring lower extremity amputations annually due to decreased limb perfusion (2). Surgical and endovascular revascularization (3) is frequently performed, but 40% of patients with critical limb ischemia (CLI) do not have a revascularization option due to extreme tissue damage and/or diffuse atherosclerotic disease (4,5). There is also a high rate of post-revascularization amputation and therefore a need to develop new tissue salvage therapy for these patients exists (6).

Minimally invasive, injectable therapies have been investigated as a promising option for treating patients with PAD. Potential therapies that utilize cells, growth factors, or gene therapies are currently in clinical trials with the goal of increasing perfusion in the ischemic limbs (7,8). Cell therapy has however been plagued by poor cell retention and survival, as well as the viability and expense issues surrounding a living product. Growth factor therapies have suffered from poor retention of therapeutic proteins in the tissue, and gene delivery results have not translated to clinical benefit (9). While injectable biomaterial alone approaches have been explored to treat ischemic cardiac muscle (10,11), they have not been extensively explored for treating the ischemic skeletal muscle associated with PAD. When designed appropriately, injectable biomaterials can be employed to create new scaffolds that recruit endogenous cells to repair damaged tissue.

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Our group previously developed an injectable hydrogel derived from decellularized porcine skeletal muscle extracellular matrix (SKM) (12). Preliminary histological analysis within the local region of the injected biomaterial in a mild rodent hindlimb ischemia model suggested that this acellular approach has the potential to not only stimulate vessel growth, but also could aid in treating the muscle atrophy associated with PAD (12). However, the global effect on neovascularization and muscle remodeling, as well as functional perfusion, which is a mainstay of translational studies for PAD, were not assessed. In the present study we

ABBREVIATIONS AND ACRONYMS

CLI = critical limb ischemia

ECM = extracellular matrix

hUC = human umbilical cord matrix

LASCA = laser speckle contrast analysis

PAD = peripheral artery disease

SKM = skeletal muscle matrix