Regenerative Effects of Extracellular Matrix on Skeletal Muscle Tissue Following Volumetric Muscle Loss: A Literature Review

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ABSTRACT

Numerous cases of severe tissue damage occur every day either from injuries, diseases, or surgery. Through tissue engineering, it is possible to restore muscle functionality that is lost from Volumetric Muscle Loss (VML). Through the process of decellularization, in which cells and DNA are extracted from tissues or organs, a biomaterial called extracellular matrix (ECM) can be fabricated. Bioengineered implants constructed of extracellular matrix (ECM) has shown tremendous potential in repairing VML and damaged muscle tissue. ECM bioscaffolds have been implanted into small rat models to investigate its regenerative properties and better understand the mechanisms that control the growth process. Multiple sources of evidence indicate that ECM is capable of altering the natural healing response of damaged tissue and promoting substantial proliferation of myofibrils. This review explores the regenerative effects of ECM and its abilities in recruiting myogenic progenitor cells, improving muscle function, and promoting constructive remodeling.

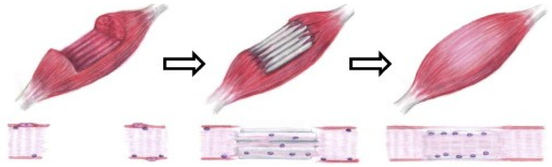


Figure 1: Demonstration of a muscle scaffold implant to treat VML.

ECM-BASED SCAFFOLDING TECHNIQUES

- After decellularization, a fully optimal ECM would closely mimic the environment of the tissue it is implanted into.
- ECM contains growth factors and myoinductive components that stimulate muscle growth and repair.

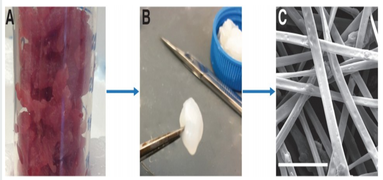


Fig. 2: Fabrication of electrospun dECM (Native tissue of a rabbit is converted into A decellularized extracellular matrix scaffold.)

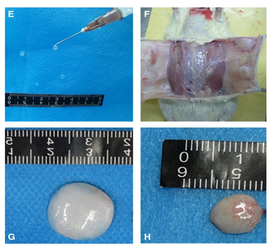


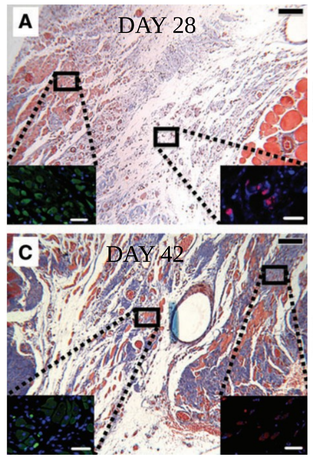
Fig. 3: Hydrogen Implantation (Comparison of solidified hydrogel 2 hours vs. 24 hours.)

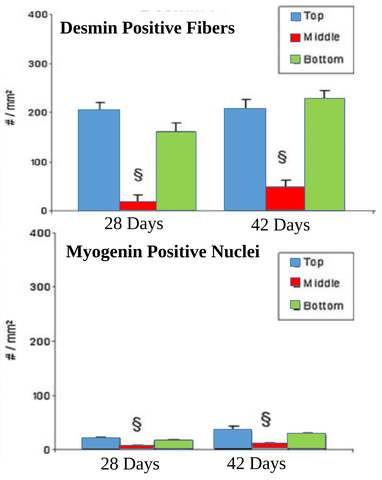
- The fabrication of electrospun dECM scaffolds allows for modulation of architecture (fiber orientation) and mechanical properties.
- The intrinsic porosity of electrospun scaffolds also makes it accessible for the exchange of nutrients and metabolic waste.
- Hydrogels maintain the components and structure of ECM while also optimizing the maximum load, compressive strength, elastic modulus, and stiffness of the hydrogel.

ANALYSIS OF ECM IMPLANTATION

ECM promotes growth of myofibrils

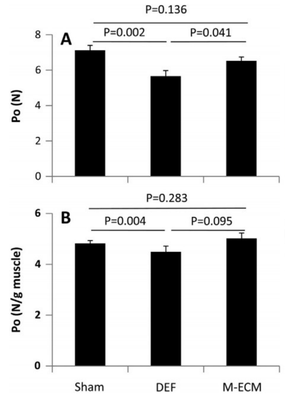
- To determine myofibril formation in an ECM implant, two biochemical components were immunostained:
  - myogenin (right box), a transcription factor involved in myogenesis and muscle repair.
  - desmin (left box), a muscle specific cytoskeleton protein.
- The amount of desmin positive fibers and myogenin positive nuclei were significantly higher in comparison between 28 days and 42 days post-recovery.





Functional improvement of ECM repaired defects

- Functional analysis was performed by measuring the peak isometric force of the nerve on the latissumus dorsi of a rat model.
- It was recorded that the absolute maximal isometric force (Po) of the mECM repair group was significantly higher than that of the non-repaired muscle group.
- There was little to no difference in absolute and normalized Po between the control group and ECM-repaired group.



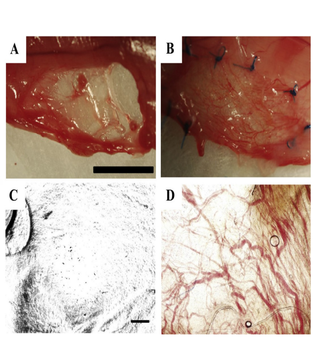
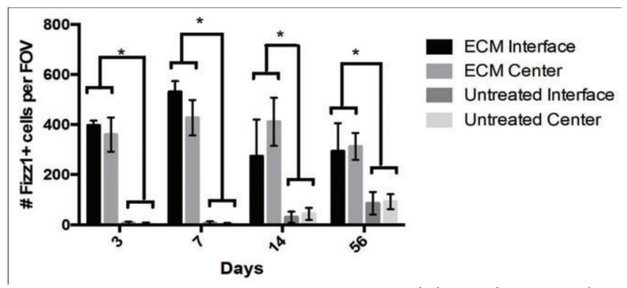
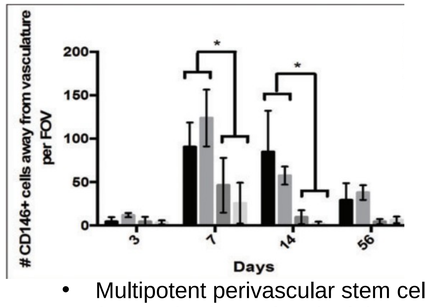


Fig 4: Comparison of non-repaired and ECM repaired muscle defect

Immunomodulation and Mobilization of Progenitor Cells





- Analyzing the pattern of macrophage activation and movement of myogenic progenitor cells caused by ECM implantation will provide insight about the specific biological reactions that result in tissue regeneration.
- M2(Fizz1 phenotype) macrophages activate myogenic progenitor cells that result in myogenesis.
- ECM bioscaffolds have a direct correlation to the increase of M2 macrophages when implanted into a mouse model of VML.
- Multipotent perivascular stem cells (PVSCs) migrated to the injury site with the ECM implantation in comparison with the untreated defect where no PVSCs were present.
- PVSCs further contribute to myofibril growth.

DISCUSSION/CONCLUSION

- The increase in myofibril formation over the course of 42 days indicates ECM is myoinductive and promotes myoblast differentiation.
- The improvement in isometric force of the rat's motor nerves in the the latissumus dorsi indicates that neurogensis took place within the defect repaired ECM.
- ECM modifies the immune response and recovery of damaged skeletal muscle by increasing myoinductive, M2 macrophage activation and PVSC recruitment.
- The mechanisms that promote constructive remodeling, restored function, and angiogenesis are still not fully understood. The addition of stem cells may be required to increase myofibril growth for larger defects.
- Further studies need to be done to fully optimize an ECM bioscaffold to produce clinically relevant volumes of skeletal muscle fiber for VML.
- Majority of in vivo studies are observed through small rodent models and have yet to be incorporated into larger mammals. The study on functionality demonstrates the potential of neurogenesis, but the dimensions of the defect does not reflect large-scale muscle damage.

FUTURE WORK

An optimized ECM scaffold will allow for full control of the architecture and tensile properties that can be adjusted for a range of muscle types. Improvement of the bioscaffold and the incorporation of stem cell therapies can allow for large-volumes of tissue regeneration and restored functionality for clinical use.

REFERENCES

1. Chen, X. K., & Walters, T. J. (2013). Muscle-derived decellularised extracellular matrix improves functional recovery in a rat latissimus dorsi muscle defect model. *Journal of Plastic, Reconstructive & Aesthetic Surgery, 66*(12), 1750-1758. doi:10.1016/j.bjps.2013.07.037
2. Corona, B. T., Wu, X., Ward, C. L., Mcdaniel, J. S., Rathbone, C. R., & Walters, T. J. (2013). The promotion of a functional fibrosis in skeletal muscle with volumetric muscle loss injury following the transplantation of muscle-ECM. *Biomaterials, 34*(13), 3324-3335. doi:10.1016/j.biomaterials.2013.01.061
3. Dziki, J. L., Sicari, B. M., Wolf, M. T., Cramer, M. C., & Badylak, S. F. (2016). Immunomodulation and Mobilization of Progenitor Cells by Extracellular Matrix Bioscaffolds for Volumetric Muscle Loss Treatment. *Tissue Engineering Part A, 22*(19-20), 1129-1139. doi:10.1089/ten.tea.2016.0340
4. Fu, Y., Fan, X., Tian, C., Luo, J., Zhang, Y., Deng, L., . . . Lv, Q. (2016). Decellularization of porcine skeletal muscle extracellular matrix for the formulation of a matrix hydrogel: A preliminary study. *Journal of Cellular and Molecular Medicine, 20*(4), 740-749. doi:10.1111/jcmm.12776
5. Merritt, E. K., Cannon, M. V., Hammers, D. W., Le, L. N., Gokhale, R., Sarathy, A., . . . Farrar, R. P. (2010). Repair of Traumatic Skeletal Muscle Injury with Bone-Marrow-Derived Mesenchymal Stem Cells Seeded on Extracellular Matrix. *Tissue Engineering Part A, 16*(9), 2871-2881. doi:10.1089/ten.tea.2009.0826
6. Smoak, M. M., Han, A., Watson, E., Kishan, A., Grande-Allen, K. J., Cosgriff-Hernandez, E., & Mikos, A. G. (2019). Fabrication and Characterization of Electrospun Decellularized Muscle-Derived Scaffolds. *Tissue Engineering Part C: Methods, 25*(5), 276-287. doi:10.1089/ten.tec.2018.0339

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