JOHNS HOPKINS
WHITING SCHOOL
of ENGINEERING

Modeling Approaches to Cell and Tissue Engineering

Skeletal Muscle Regeneration. The Use of Biomimetic Scaffolds

Skeletal Muscle Regeneration. The Use of Biomimetic Scaffolds

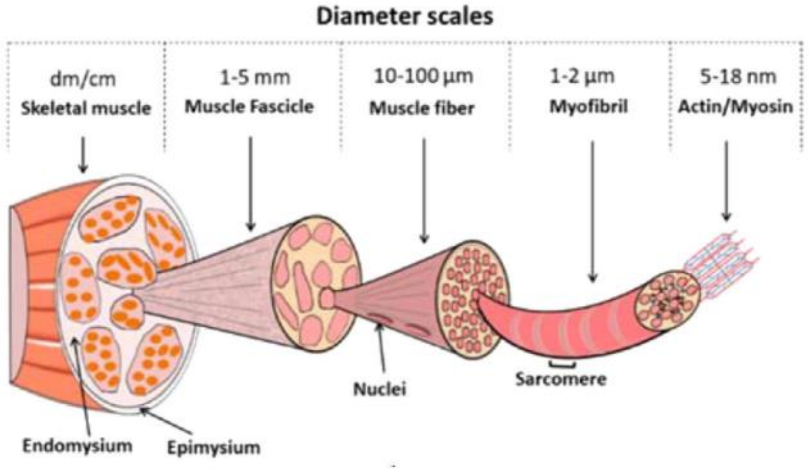
Alexander A. Spector (From B. Lindgridge et al., 2021)

1. Volumetric Muscle Losses. Medical Problems
2. Biomimetic Scaffolds
3. Cells Used in Skeletal Muscle Regeneration
4. Decellularized Scaffolds
5. Nanofibers
6. Hydrogels
7. Electroconductive Scaffolds

Scaffold Biocompatibility

The ideal scaffold recapitulates the native skeletal muscle microenvironment; anisotropic three-dimensional (3D) scaffolds provide a biomimetic microarchitecture, topographical cues, along with cellular adhesion molecules that are necessary for muscle progenitor cells to differentiate and organize into functional muscle tissue. Scaffold biocompatibility is a key requirement to avoid foreign body type reactions, with tissue fibrosis, scaffold encapsulation and resultant graft failure. The mechanisms of biocompatibility are incompletely understood, however, experience with implanted medical devices and organ transplantation has demonstrated the importance of autologous organic materials or inert inorganic materials. Replicating the mechanical properties of the native ECM is also important, several studies have demonstrated that optimal myogenesis occurs on scaffolds with muscle like stiffness.

Skeletal Muscle Components at Different Scales and of Volumetric Muscle Loss



Different scales of skeletal muscle components. Histological sample from a porcine model of VML injury was created through surgical excision e peroneus tertius muscle, with histological samples taken at12 weeks after injury. Significant fibrosis infiltrating into native muscle is seen. Masson’s Trichrome stained sample (Connective tissue is blue; nuclei are purple; skeletal muscle fibres are red).

Clinical Need in Scaffolds for Treatment of Volumetric Muscle Loss

Skeletal muscle is capable of regeneration following minor damage, more significant volumetric muscle loss (VML) however results in permanent functional impairment. Current multimodal treatment methodologies yield variable functional recovery, with reconstructive surgical approaches restricted by limited donor tissue and significant donor morbidity. Tissue- engineered skeletal muscle constructs promise the potential to revolutionize the treatment of biocompatible tissue scaffolds, including recent developments with electroconductive materials. Secondly, we review the progenitor cell populations used to seed scaffolds and their relative merits. Thirdly we review in vitro methods of scaffold functional maturation including the use of three-dimensional bioprinting and bioreactors. Despite significant advances in areas, such as electroactive scaffolds and three-dimensional bioprinting, along with several promising in vivo studies, there remain multiple technical hurdles before translation into clinically impactful therapies can be achieved.

Progenitor Cell Populations Used in Tissue-Engineered Skeletal Muscle Constructs

Cell Type	Origin	Advantages	Disadvantages
Satellite Cells	Skeletal Muscle	Native stem cell for muscle regeneration in vivo, Efficient differentiation, Widely used in skeletal muscle tissue engineering	Invasive collection method, Low yield isolation processes, Senescence causes reduced myogenic potential after expansion in culture
Murine C2C12 Myoblasts	Immortalized Murine Myoblast Cell Line	Rapid proliferation, Efficient Differentiation, Commercially available	Immunogenicity in vivo

Scaffold Materials: Decellularized scaffolds

Decellularized scaffolds are derived from xenogeneic, allogenic or autogenic skeletal muscle tissue. Once decellularization has removed cellular material, the remaining ECM retains the native 3D microstructure, molecular composition and growth factors that support skeletal muscle regeneration. Decellularized scaffolds have the advantage of a ready-made, tissue-specific ECM with the appropriate microarchitecture and molecular composition. Their ability to natively support myogenesis and angiogenesis is a significant advantage over synthetic scaffolds which require extensive development to gain similar characteristics.

Scaffold Materials: Nanofibers

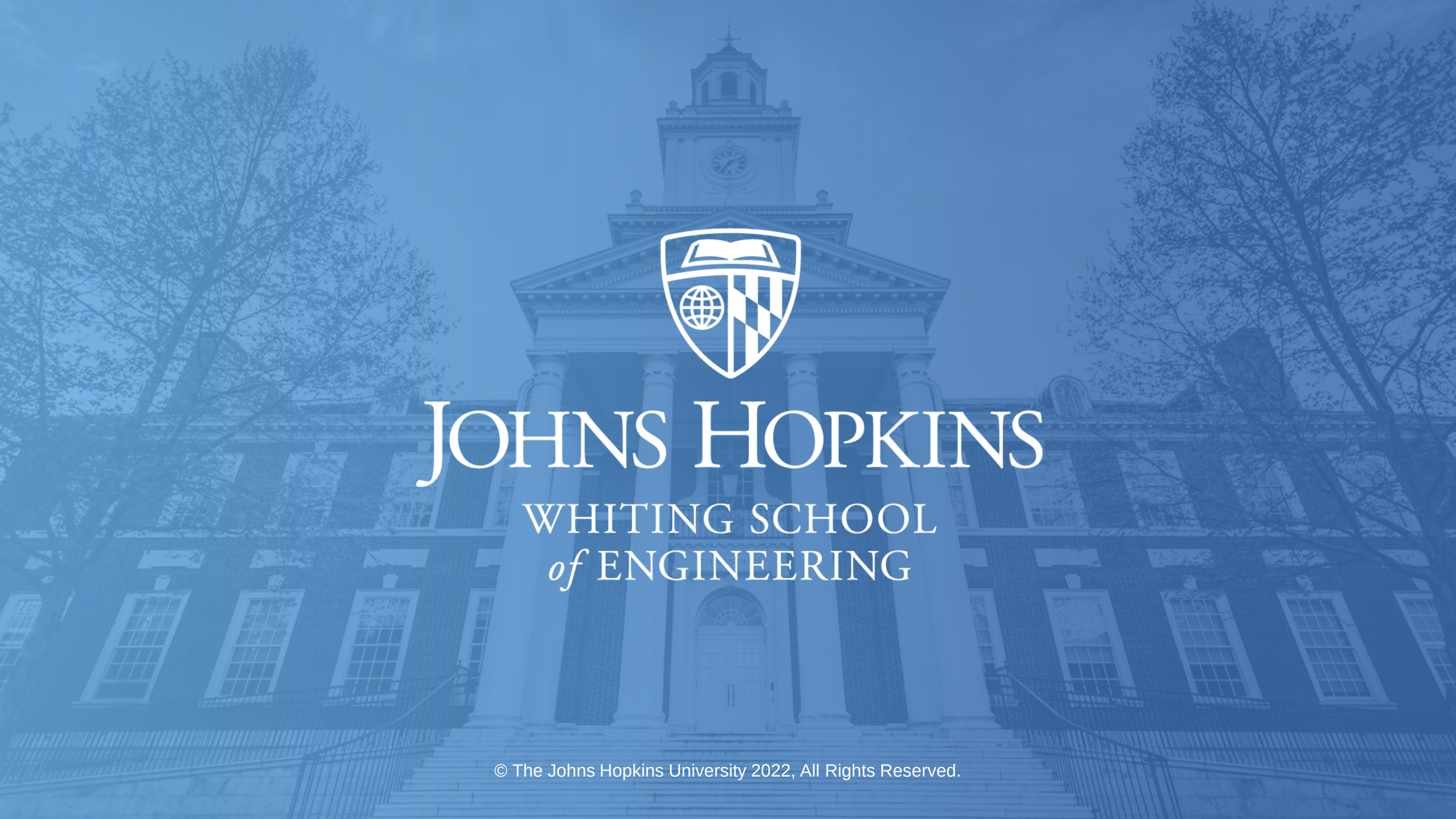
Nanofibrous scaffolds are defined as a mesh of nanoscale (0–100 nm) synthetic fibers, these can closely mimic the architecture of the native ECM. Nanofibers can be manufactured via several methods including thermal cycling and phase separation, or electrospinning. Electrospinning is widely used in skeletal muscle tissue engineering given the ability to produce anisotropic, geometrically aligned nanofibers capable of guiding the formation of aligned myofibers. Nanoscale materials can be utilized to modify the properties of nanofiber scaffolds in unique ways to improve mechanical properties, wettability, cellular adhesion, cellular differentiation and electroconductivity.

Scaffold Materials: Hydrogels

Hydrogels are a family of hydrophilic polymers with a high-water content consisting of either natural or synthetic materials. Natural hydrogels consist of materials, such as collagen, fibrin, chitosan and hyaluronic acid; they are biodegradable, but have limited mechanical strength and can provoke an immune response in vivo.Synthetic hydrogels, such as polyethylene glycol have superior mechanical properties that can be tailored more readily however, as they inherently lack biological molecules, they require modification to support cell adhesion, differentiation and viability. Hydrogels are also highly suitable for the entrapment of cells and biomolecules, such as growth factors, that promote cellular survival, myogenic differentiation and angiogenesis within the hydrogel.

Scaffold Materials: Electroconductive Scaffolds

In vivo, skeletal muscle receives motor neuron innervation which, via the neuromuscular junction, causes cell membrane depolarization and myofiber contraction. This electrochemical stimulation not only affects mature myofiber function but is also necessary for normal myoblast differentiation during embryonic development. Electroactive scaffolds have been developed through the incorporation of carbon nanotubes, graphene, metals and conductive nanopolymers to make novel nanocomposites. Carbon nanotubes are of interest as they are renowned for their remarkable strength, elasticity and electrical conductivity.


JOHNS HOPKINS
WHITING SCHOOL
of ENGINEERING