1. What are the major groups of stem cells used to seed the scaffold? What are their advantages and disadvantages?

   The major groups of stem cell populations used to feed the scaffold are:

Cell Type	Advantages	Disadvantages
Satellite cells	- They are the native stem cells used for skeletal muscle regeneration in vivo and widely used in skeletal muscle tissue engineering - They can be efficiently differentiated (up to 50 times in vitro)	- They require invasive collection method - They can lose stem cell potency in ex vivo - Cellular senescence during extended culture expansion leads to a decline in the cells' myogenic potential
iPSCs	- They are capable of unlimited self-renewal - They can differentiate into myotubes and satellite-like cells - They can integrate with existing muscle and vascularize in vivo	- Cellular reprogramming is characterized by very low efficiency. - There is a significant risk of tumor formation.
MSCs	- They have a high proliferative potential - Bone MSC is a high yield process - Umbilical MSC collection process is non-invasive	- Lower myogenic differentiation potential compared to satellite cells - Bone MSC collection is painful and invasive - Low availability of autogenic umbilical MSCs

2. List the main materials used for skeletal muscle regeneration?
   The scaffolds used for skeletal muscle regeneration are the following:
   - Decellularized scaffolds are derived from xenogeneic, allogenic or autogenic skeletal muscle tissue.
   - Nanofibrous scaffolds are defined as a mesh of nanoscale synthetic fibers closely mimicking the architecture of the native ECM. Nanofibrous scaffolds can be made of natural materials, such as Collagen, or artificial polymers, such as Polycaprolactone (PCL).

- Hydrogels are a family of hydrophilic polymers consisting of natural hydrogels, such as Collagen, fibrin, chitosan, and hyaluronic acid, or synthetic hydrogels, such as polyethylene glycol (PEG).
- Electroconductive scaffolds incorporate carbon nanotubes, graphene, metals and conductive nanopolymers.

3. What is the level of the scaffold stiffness that provides the most significant myogenesis?
   Optimal myogenesis occurs at scaffold stiffness comparable to muscle stiffness (typically a Young's modulus around 12 kPa).

4. What are the medical areas whose experience is important for further use in muscle regeneration?
   - Volumetric muscle loss (VML) is common in open fractures, limb salvage, and combat-related injuries, so orthopedic/trauma surgeons' experience with severe musculoskeletal defects is critical.
   - The standard of care includes extensive plastic and reconstructive surgery to perform free-flap transfers, cover donor-site morbidity, and perform functional muscle transfers. Rehabilitative physiotherapy is also essential for restoring muscle function.
   - To scale the production of tissue-engineered constructs, it is important to develop efficient, low-cost, and standardized processes. This includes ensuring safety and clinical efficiency, which necessitates expertise in medical industrial manufacturing and regulatory experience to navigate FDA and EMA regulations.

5. What are the features of skeletal muscle that the scaffolds reproduce?
   Different types of scaffolds can support cellular survival, promote myoblast proliferation, differentiation, and functional maturation, facilitate myofiber differentiation, produce aligned myofibers, and enhance myocyte functional maturation, regeneration, and contractility. They can also facilitate angiogenesis.