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Commentary: benefits and challenges of cellularized scaffolds for treatment of volumetric muscle loss

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Abstract: Bulk local tissue loss may be amenable to scaffold based therapies. Discrete areas can usually be filled in with composite scaffolds, which can then conform and integrate to host tissue. Many preclinical studies have demonstrated excellent efficiency in soft tissue reconstitution with acellular scaffolds, but tissue function and clinical translation have not been fully realized. Newer scaffolds carry growth factors and are structured to mimic host tissue architecture, to promote host tissue regeneration and scaffold ingrowth. Despite these considerable advances, the addition of cellular components, often in the form of stem or progenitor cells, can promote further return of tissue function that scaffolds alone. Inclusion of any cell types, however, results in increased costs, potential delay treatments, and vastly increased regulatory hurdles. Herein, we explore the benefits and concerns with the use of cells in scaffold-based tissue engineering, with a focus on tissue defects in muscle and clinical application.

Keywords: scaffolds; tissue regeneration; stem cells; tissue defects; volumetric muscle loss; induced pluripotent stem cells; growth factors

The central goal of tissue engineering is to restore or regenerate tissue, commonly following injury. Volumetric muscle loss (VML) are areas of composite, discrete muscle loss, often a result of traumatic injury. When these injuries comprise at least 20% of the total muscle mass, these areas heal primarily with fibrosis and minimal muscle regeneration [1]. As such, strength restoration is substantially reduced as compared to normal. As many of these injuries involve lower extremities following motor vehicle accidents, patients further suffer from a loss of ambulation and, subsequently, independence. Therapeutic options for VML remain limited. Non-invasive therapies, such as physical rehabilitation, improve function past an injured baseline but do not allow patients to approach levels of pre-injury function [2]. Often, operations for VML involve wound coverage with skin grafts or non-functional,

fasciocutaneous flaps. Though important for wound closure and overall patient recovery, these operations do not promote functional muscle recovery. Functional skeletal muscle transfer is a potential operation to improve muscle recovery but is a limited therapeutic option as (1) not all patients are candidates for the procedure, (2) there is a resultant loss of strength at the site of the donor muscle, and (3) there is incomplete or marginal strength restoration [2]. Taken together, tissue engineering techniques may be of great benefit to restore function in patients suffering from VML.

Our group has demonstrated that acellular scaffolds can indeed improve muscle functional recovery following VML injury in rodents, especially when accompanied with regimented exercise [3]. Despite this improved recovery, we acknowledge that further improved muscle regeneration and strength restoration may be necessary to provide substantial benefit to patients. The success of acellular therapies depends upon the activation, migration, and differentiation of muscle satellite cells, which may not be sufficient for regenerating large muscle defects [4]. The addition of cells has been explored as an alternative solution to acellular scaffolds. Among various cell options, our studies confirmed that induced pluripotent stem cell-derived myogenic progenitor cells (iPSC-MPC), greatly enhanced functional muscle recovery in our standardized VML preclinical model [5]. Human iPSC-MPCs can engraft and contribute to repair in our immunocompromised mouse model, suggesting a role for myogenic cells in clinical treatment of VML injuries (Figure 1). However, utilization of cells in translational therapies are significantly more challenging than using them in pre-clinical models.

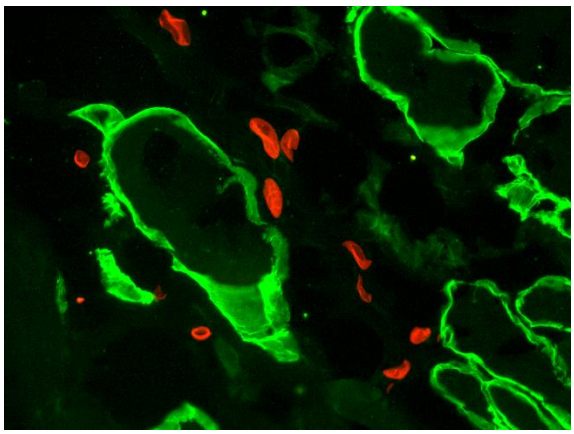

Figure 1. Human iPSC-MPCs (labeled red) demonstrate engraftment in a model of volumetric muscle loss within an immunocompromised mouse. Red-Human lamin, Green-Mouse Laminin, cross section of gastrocnemius muscle.

iPSC-derived cell usage circumvents some of the risks regarding stem cell transplantation by being autologous, thereby not requiring immunosuppression. Still, the use of autologous iPSCs for clinical therapy comes with considerable challenges [6]. In the case of traumatic VML injury, skin harvest, isolation of terminally differentiated cells, re-programming them into iPSCs, and differentiating them into myogenic precursors, and then embedding them into a scaffold may take in the order of months, thus delaying patient care. Autologous

cell delivery would require this process to be carried out, completely, for every patient. Similar therapies are performed for keratinocyte expansion in burn patients, although the biological methods and rigor for proliferating keratinocytes are much less than for iPSC-MPCs [7]. Additionally, this process is expensive and difficult to automate as samples come in from different patients at different times. Utilization of allogenic cells can be much better automated, reducing costs and providing therapies in a timely fashion. However, unless large numbers of cell lines are available and antigenicity properly understood, the use of allogenic cells may require immunosuppression, even during a transient period, which greatly increases risk of morbidity and even mortality in many patients. As such, methodologies to improve cost and delay in autologous iPSC delivery are an area of intense research.

Another important challenge in cell-based therapies is sustaining their survival *in vivo*. This requires their access to oxygen and nutrients that are brought to cells via vasculature [8]. While most researchers believe that to achieve this goal, scaffold vasculature should be properly connected to the host circulatory system, recent studies have shown that even without surgical anastomosis the presence of vasculature templates can support cell survival and engraftment.

The Food and Drug Administration (FDA) has set forth guidelines for the incorporation of stem cells into therapeutic devices [9]. Amongst the regulations required by the FDA for allogenic transplants are pre-clinical studies, safety profiles, qualification of vendors and donors, details of raw material sourcing, and internal validation metrics. Sources of isolation must be defined and tested for pathogens; commonly peripheral blood or skin are used to generate iPSCs. In addition, retroviral re-programming of somatic cells may come under scrutiny, as non-specific changes to the cellular genome may occur as well. Genetic reprograming techniques must be further refined. Lastly, robust characterization of the developed cells, such as iPSC-MPCs, must be carried out. This includes tests of sterility, proliferative rate, purity, genomic instability, and potency. Each of these factors are critical in developing translational stem cell therapies, but all add additional cost and time for these therapies to be clinically available.

Despite these substantial challenges, the utilization of cellular materials and iPSCs, in particular, is a growing area of research development for generation of myriad tissues. The use of these cells can efficiently and robustly replace lost tissue in a manner not possible with conventional means. For VML, it greatly augments recovery with scaffold placement and promotes reconstitution of lost muscle. Developing cells for routine clinical will require streamlining of manufacturing and comprehensive analysis methods to ensure safety and efficacy. These efforts seem well worthwhile, as cellular therapies in combination with scaffolds can revolutionize the care we can provide our patients.

Conflicts of interests

Indranil Sinha reports a relationship with InPrint Bio LLC that includes: equity or stocks. Ali Tamayol reports relationships with InPrint Bio LLC and 3D PenBone LLC that include: equity or stocks.

Authors' contribution

Drs. Sinha and Tamayol both participated in original draft preparation, review, and editing. All authors have read and agreed to the published version of the manuscript.

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