Review Article

Interface Tissue Engineering: Next Phase in Musculoskeletal Tissue Repair

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Abstract

Increasing incidence of musculoskeletal injuries coupled with limitations in the current treatment options have necessitated tissue engineering and regenerative medicine- based approaches. Moving forward from engineering isolated musculoskeletal tissues, research strategies are now being increasingly focused on repairing and regenerating the interfaces between dissimilar musculoskeletal tissues with the aim to achieve seamless integration of engineered musculoskeletal tissues. This article reviews the state-of-the-art in the tissue engineering of musculoskeletal tissue interfaces with a focus on Singapore's contribution in this emerging field. Various biomimetic scaffold and cell-based strategies, the use of growth factors, gene therapy and mechanical loading, as well as animal models for functional validation of the tissue engineering strategies are discussed.

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Introduction

As in all developed societies, Singapore has a significant ageing population (about 20% of its population being over the age of 55 years) which is prone to degenerative musculoskeletal disease.[1] Musculoskeletal injuries are also common among the younger population, resulting from strenuous physical activities during sports and national military service that all young Singaporean men are required to perform. Military training is known to more likely result in orthopaedic injuries compared to sports activities.[2]

Limitations in the currently available treatment modalities for severe orthopaedic injuries such as allogeneic/autogeneic tissue grafts or prosthetic implants have paved the way for tissue engineering and regenerative medicine-based approaches that offer the possibility of a cure, not a treatment, and for almost all diseased or injured tissues and organs to be potentially regenerated. Musculoskeletal tissue engineering research has been primarily focused on injuries and diseases of tissues such as the bone, cartilage, tendon and ligament, while repairing and regenerating of musculoskeletal tissue interfaces have been relatively unaddressed. Musculoskeletal tissue interfaces connect dissimilar tissues such as the bone, cartilage, tendon, ligament and muscle and help in the efficient transfer of load between these tissues, allowing for optimal motion and stability. The interfacial tissues typically possess physical, biochemical, biological properties and cellular composition that are distinct from the adjoining tissues that they connect, often with a gradual transition in mechanical properties to minimise stress concentrations at the interface. However, these interfaces are prone to injury and standard surgical repair methods fail to regenerate the intricate interface, compromising graft stability and long-term clinical outcome.

Musculoskeletal tissue engineering research in Singapore

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is primarily conducted in the National University of Singapore (NUS), the A*STAR research institutes (i.e. Institute of Bioengineering and Nanotechnology, and Institute of Molecular Biology), and the Nanyang Technological University. In this article, we review the state-of-the-art in the tissue engineering of musculoskeletal tissue interfaces with a focus on NUS's contribution in this emerging field. The key tissue engineering strategies, potential challenges and future directions that can help achieve seamless integration of engineered musculoskeletal tissues are also discussed.

Musculoskeletal Tissue Interfaces

The functionally important musculoskeletal tissue interfaces are those that exist between tendon/ligament and bone (enthesis), bone and cartilage (osteochondral and meniscus-bone interface) and the muscle-tendon (myotendionous) interface. The soft tissue to implant interface in metallic and ceramic orthopaedic implants is another relevant interface where interfacial engineering approaches have been used.[3,4] The fibrous or fibrocartilage enthesial interface between tendon/ligament and bone has a multiphasic structure transitioning from soft fibrous tendon/ligament tissue, through uncalcified and calcified fibrocartilage zones, to hard bone tissue. Such an interface is crucial for load transfer between these tissues without the formation of stress concentrations and is responsible for the high insertional strength and avulsion resistance of tendons and ligaments.[5] The osteochondral interface at the articular end of a bone similarly comprises of calcified cartilage that has vertical collagen fibers continuous with the deep zone of calcified hyaline cartilage on one side and interdigitating with subchondral bone on the other side. This structural arrangement reduces lateral tissue strain and stress concentrations preventing cartilage cracking, and also increases the interfacial area providing tighter adherence. Such increase in interfacial area is also observed at the myotendinous junction due to excessive folding of myofibre cell membranes that also reduces the loading angle, further protecting the interface.

Musculoskeletal Interface Tissue Engineering

With the emergence of the concept of "functional tissue engineering", it is now understood that successful tissue regeneration would require the engineered tissue to possess similar physical and physiological characteristics as the target tissue. This is particularly relevant for interfacial tissue engineering where structure and function are critically coupled. Thus, interfacial tissue engineering approaches aim to replicate the micro- and nano-structure of the interface in the form of advanced scaffolds, or replicate the complex cellularity or biochemical composition of the interface through cell co-cultures and growth factor gradients. These strategies are focused either to generate the interface alone, or to generate it as part of an integrated multi-organ complex, such as a bone-ligament graft or an osteochondral plug. The approaches can be roughly grouped into the following categories:

- Scaffold-based strategies: multi-phased scaffolds;
- Cell-based strategies: stem cells and co-cultured cells;
- Growth factors and gene therapy;
- Mechanical loading in bioreactors.

Scaffold-based Strategies

A scaffold is essentially an engineered replacement of the native extracellular matrix (ECM), and the ideal scaffold would be one that biomimics the physical and physiological characteristics of the target tissue. Thus, complex scaffolds have been developed by incorporating a gradient or different phases, suitable for engineering tendon/ligament, cartilage and bone, into different zones of the scaffold for interfacial tissue engineering. As an example, the ligament phase has been made up of a PLGA fibrous mesh, the interfacial phase of PLGA microspheres and the bone phase of sintered

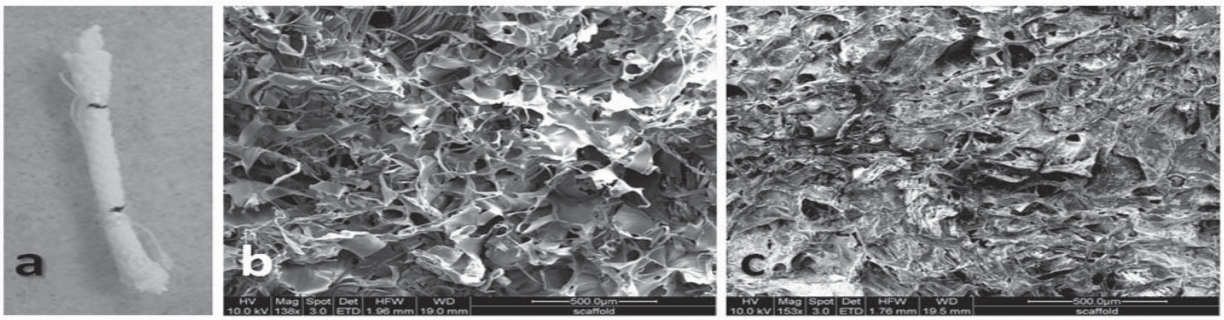

Fig. 1. (a) A rolled up triphasic silk scaffold, and (b) the SEM ultrastructure of its central sponge-coated ligament region and (c) sponge + HA-coated bone regions.

composite PLGA and bioactive glass microspheres.[6,7]

Biphasic/Triphasic Scaffolds: In order to regenerate the tendon/ligament-bone interface to facilitate functional graft-to-bone integration, scaffolds have been designed in the form of multiphasic structures with separate phases suitable for engineering tendon/ligament and bone. A tissue engineered bone-ligament-bone (BLB) graft incorporating the ligament-bone interface has been developed by using a triphasic silk scaffold with zones for ligament, cartilage and bone tissues. The triphasic 3D silk scaffold was fabricated by coating a knitted silk microfibrous scaffold with lyophilised silk sponge and osteoconductive hydroxyapatite on the two bone ends (Fig. 1). Such scaffolds have been differentially seeded with multiple cell lines appropriate for regenerating the different target tissues (bone, fibrocartilage or ligament), and are expected to be more promising than ligament-alone constructs for long-term repair of severely injured ligaments. Multiphasic scaffolds have also been developed using sintered polymer-ceramic composites for ligament-bone and osteochondral tissue engineering.[7,8]

Cell-based Strategies

Progenitor/Stem cells: Several cell-based approaches have been used to regenerate orthopaedic interfaces. It is hypothesised that multipotent stem cells, when placed in an appropriate scaffold providing a permissive and instructive microenvironment, could regenerate specific interfaces. Bone marrow derived mesenchymal stem cells (MSCs) have been shown to enhance the integration of tendon grafts in bone tunnels in rabbit models.[9,10] MSC-enhanced grafts were shown to develop an interface with distinct zones of tendon, uncalcified fibrocartilage, calcified fibrocartilage, and bone, which was absent in the MSC-free control (Fig. 2). Successful regeneration of the tendon-bone interface played a crucial role in improving the mechanical properties of the construct as the MSC-enhanced grafts displayed better mechanical properties compared to MSC-free controls.[10]

Co-cultured cells: Since the interfacial regions typically have a mix of different cell types, tissue engineering strategies have used co-cultures of relevant cells on scaffolds to engineer the tissue interface. Tong et al used a co-culture of osteoblast and chondrocytes on a 3D polymer scaffold fabricated via fused deposition modeling in an attempt to engineer an osteochondral construct.[11] In a separate study, sequential co-culture of osteoblasts and chondrocytes in 3D culture showed that co-culture interactions modulated both cell phenotypes.[12] Similarly, fibroblast-osteoblast co-cultures have been shown to modulate cell phenotypes and possibly generate a phenotype of interfacial fibrocartilage

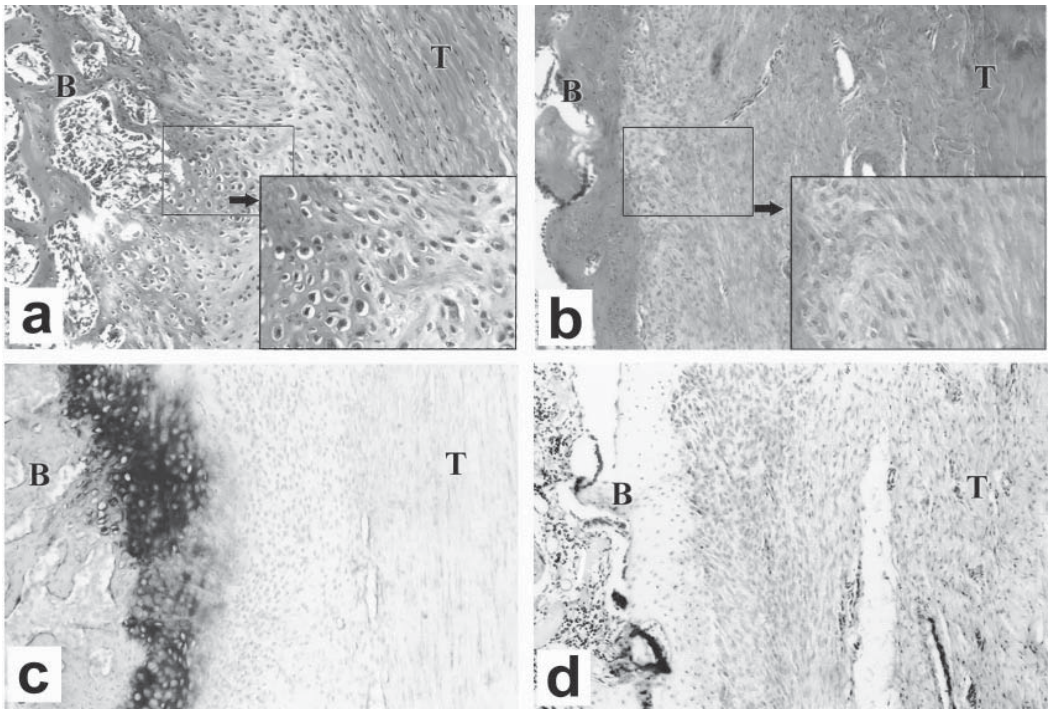

Fig. 2. The effects of MSCs in the biological restoration of tendon (T)-to-bone (B) insertion: (a,b) histology and (c,d) collagen type II immunostaining showed that the MSC-treated group (a,c) could regenerate the fibrocartilaginous interface which was absent in the control group (without MSCs; b,d), 4 weeks after surgery (100x).[10]

cells via cellular transdifferentiation.[13]

Other approaches have attempted to mimic the developmental anatomy and organogenesis of orthopaedic interfaces wherein interfacial tissues are laid down between different developing tissues. Based on the hypothesis that progenitor/stem cells co-cultured between appropriate differentiated cells would cross-talk via intercellular signals and paracrine factors to differentiate into interfacial tissues,[14-16] several co-culture based approaches have been attempted. Bilineage co-cultures established between MSCs and osteoblasts/ligament cells had cells communicating effectively via functional gap junctions and showed chondrogenic differentiation of the co-cultured MSCs (Fig. 3). A trilineage co-culture of MSCs, osteoblasts and ligament cells, established on a triphasic scaffold to generate a BLB construct, similarly showed cells proliferating on the 3D scaffold, with fibrocartilaginous differentiation of MSCs demonstrated by upregulated chondrogenic gene markers (Sox9, collagen type-2 and aggrecan) in the interfacial region of the scaffold after 3 weeks. Such tissue engineered BLB grafts would possess a collagenous ligament mid-substance as well as bone ends with intervening ligament-bone interfaces.[17]

Growth Factor and Gene Therapy-based Strategies

Growth factor: Growth factors play an important role during tissue development and repair, and various growth factors have been used to stimulate cell proliferation, differentiation and matrix deposition in musculoskeletal tissue engineering.[18-20] Bone morphogenetic protein-2 (BMP-2),[21-23] transforming growth factor-β1 (TGF-β1)[24] and platelet-derived growth factor (PDGF-BB)[25] have been shown to accelerate interfacial healing and enhance the insertional strength of tendon grafts. BMP-2 has also been observed to promote fibrocartilage zone formation at the tendon-bone interface in a mice model.[26] Local application of granulocyte-colony stimulating factor (G-CSF)—incorporated gelatin significantly increased bone-tendon interface strength via enhanced angiogenesis and osteogenesis in a canine model.[27] Similarly, TGF-β1[28] and basic fibroblast growth factor (bFGF)[29] have been used to generate fibrocartilage and improve cartilage integration in osteochondral tissue engineering.[28,29]

Since growth factors typically have short half lives, it is often necessary to provide frequent culture media growth factor supplementation in in vitro applications or local injections of growth factors with supra-physiologic doses in vivo in order for growth factor implementations to be effective. Repeated administration, in both the in vitro and in vivo contexts, can be avoided by controlled delivery of the bioactive growth factor from the scaffolds.[30] It is therefore critical to develop methods for incorporating various growth factors into the scaffold system without affecting their potency. Various incorporation methods have been developed and the growth factor binding efficiency and elution profile have been characterised. Specifically, bFGF has been non-covalently bound and delivered via a collagen gel onto porous 3D polycaprolactone (PCL) scaffolds.[31] Similarly, BMP-2 has been covalently tethered onto hyaluronic acid and delivered via a poly (ethylene glycol)-based photopolymerisable hydrogel to improve tendon-bone insertion in rabbits.[23]

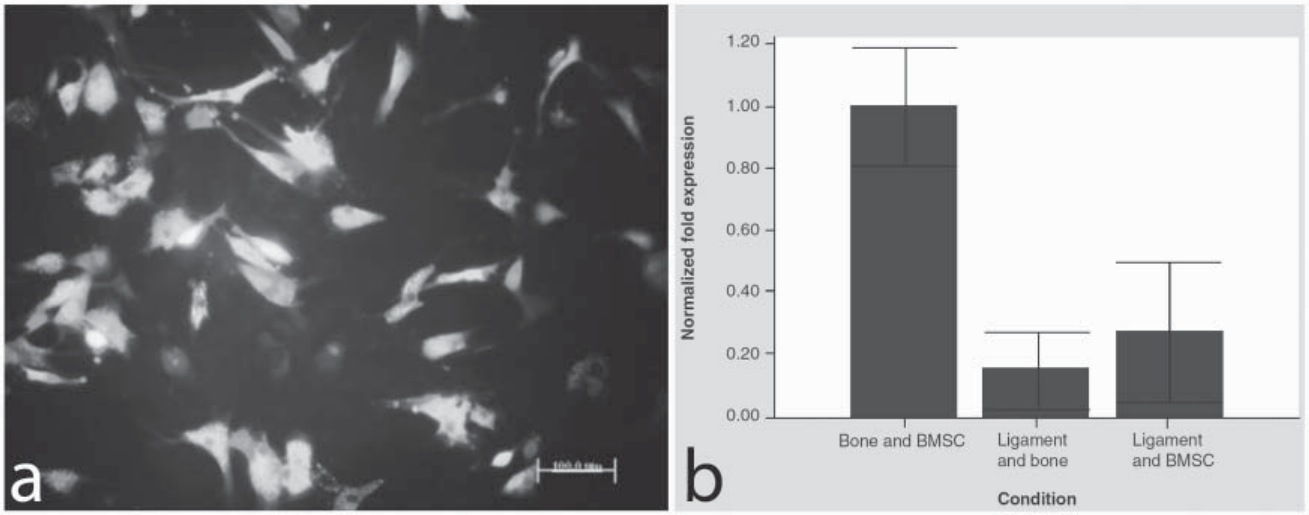

Fig. 3. (a) Confocal image showing effective co-culture of ligament cells and MSCs with establishment of functional gap junctions (dye transfer assay), and (b) RT-PCR analysis showing low expression of type II collagen in ligament/bone cell co-culture and higher expression in MSC co-cultures[17] (Adapted from Regenerative Medicine 2010;5:221-29 with permission of Future Medicine Ltd).

Gene therapy: To ensure sufficient bioavailability of the growth factors to the regenerating tissues, scaffolds need to be loaded with supra-physiologic concentrations of the growth factors. Gene transfer, wherein genetic material is delivered into target cells using viral or non-viral vectors to alter their functions, can also be used to deliver prolonged and high concentrations of growth factors at the repair site. TGF-β1[32] and BMP-2[22] transfected cells have been shown to enhance meniscus and tendon-bone tissue repair. Sustained BMP-2 gene expression for up to 6 weeks has been demonstrated after retroviral transfection of autologous tendon grafts, aiding in bone-ligament interface regeneration with significantly improved mechanical properties.[22]

Gene silencing through RNA-interference and short-interfering RNA (siRNA) is another promising technique whereby expression of target genes can be decreased, providing a valuable tool for promoting and directing the growth of functional tissues for tissue engineering applications.[33] While antisense gene therapy to down-regulate expression of decorin and collagen type V has been used to improve collagen type I fibrillogenesis and healing of tendon/ligament defects,[34,35] a clearer understanding of gene expression at various orthopaedic interfaces could open possibilities to apply gene silencing technologies for interface healing.

Mechanical Loading in Bioreactors

Bioreactors providing mechanical stimulation in the form of cyclic tension or compression have been used to condition and improve cellular orientation and matrix organisation in engineered tissues. Mechanical stimulation can direct stem cell differentiation and the beneficial effects of cyclic tensile loading in tendon/ligament tissue engineering and compressive loading in cartilage/bone tissue engineering have been demonstrated in several studies.[36-40] Engineering of complex tissues such as the orthopaedic interfaces, where different components may require different mechanical stimulation, demand the usage of advanced bioreactors that can better mimic the anisotropic stresses needed at these interfaces. For instance, fibrocartilaginous enthesis are subjected to simultaneous compression and tension under physiological conditions and a bioreactor for engineering enthesis should be able to provide a combination of compression and tension to the developing construct (Fig. 4).[41,42]

Current In Vivo Studies for Musculoskeletal Interface Tissue Engineering

Tissue engineering constructs developed using in vitro studies should be tested in in vivo models for functional assessment and validation. The in vivo host environment

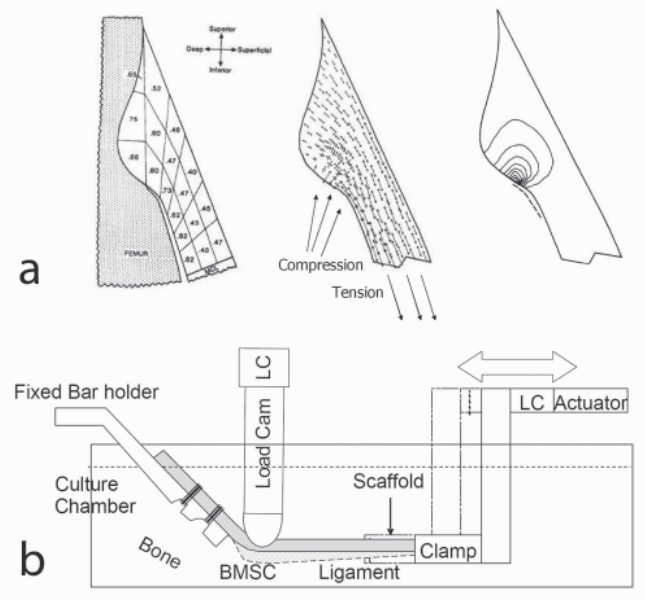

Fig. 4. (a) Physiological load distribution at the enthesis and (b) a bioreactor design for proving simultaneous compression and tension for enthesis tissue engineering.[41,42]

is expected to induce development of neo-interface structure under physiological conditions, bringing construct development closer to clinical reality. The animal models used should also be carefully designed to effectively assess the potential of the construct for musculoskeletal interfacial regeneration. Besides being physiologically relevant and easily reproducible, the animal model needs to be large enough to make surgery practical and allow structural and functional assessment of the repair outcome, while being reasonably inexpensive to permit studies with sufficient statistical power. Models for interfacial tissue engineering should, in addition, closely resemble the complex wound-healing environments in the orthopaedic interfaces. Orthopedic tissue engineering commonly employs animal models such as skeletally mature rabbits, pig, dogs and sheep.

Various musculoskeletal interfaces, including the osteochondral and tendon/ligament interfaces, have been developed and characterised using different animal models. The osteochondral interface has been regenerated within 5.0 to 5.5 mm deep osteochondral defects in the medial femoral condyle of adult New Zealand white rabbits.[43-45] On the other hand, the tendon/ligament-bone interface has been studied in the femoral and tibial bone tunnels with hamstring grafts[22] or in the calcaneal bone canal with grafted flexor digitorum longus tendon.[46] Various multiphased and multilineage cell-seeded scaffold constructs have also been developed and evaluated in animal models.[6] Altman et al[47] have developed a multi-region, porous knitted silk anterior cruciate ligament (ACL) graft and evaluated it in a goat model for a 12-month period, demonstrating the potential

of the scaffold for bioengineered ligament devices. A cell-based approach was demonstrated by Ma et al,[48] who reported the possibility of using engineered bone segments with ligament monolayers rolled up around the bone ends to form BLB segments.

Future Trends

In this review, an overview of the current development and various strategies for musculoskeletal interface tissue engineering have been presented. It should be emphasised that interface tissue engineering, in itself, is a critical next step after the development the specific soft tissue of interest, be it tendon, ligament or cartilage. It has become clearer over time that successful regeneration of musculoskeletal tissues is not limited to development of these tissues ex vivo, but also by effectively integrating them to the musculoskeletal tissue system in vivo. It is then that functional tissue engineering can be achieved.

Nevertheless, there exist several challenges in this critical step. A key hurdle includes the lack of a deeper understanding of the structure-function relationship of native tissue interfaces and the different mechanisms affecting the development and regeneration of these tissues. Since interfacial tissues are transitional in nature and often bear structural characteristics that change gradually between two distinct tissue types, it is challenging to provide suitable physical, chemical and biological cues that stimulate such unique structural transition. Hence, better understanding of the native interface tissue environment will certainly prove useful for the development of biomimetic scaffolds and conditioning criteria. Physiologically relevant in vivo models are also necessary for clinical translation of engineered interfacial tissues. Furthermore, with the use of multi-lineage cells being shown in reported studies to positively contribute to neo-interfacial tissue formation, it is critical that optimal cell source and the related isolation and expansion procedures be optimised and standardised for clinical implementation. Successful clinical translation will also encompass measureable clinical outcomes to ascertain the level of interface tissue regeneration after implantation of tissue engineered constructs. Other important issues relating to the clinical implementation of such technologies include clinical product fabrication and logistical arrangements, such as feasible sterilisation methods, tracking and storage.

Conclusion

In conclusion, it is becoming clear that the regeneration of musculoskeletal interfacial tissues is a critical step in achieving biological fixation for functional tissue engineering. With an increased understanding of the fundamental cellular processes involved in cell-cell and cell-matrix interactions, mechanotransduction, cellular migration and differentiation involved in organogenesis and progresses in research in the related technologies of biomarkers, bioimaging technologies and computational modelling systems, researchers can now study the functional anatomy and physiology at orthopaedic interfaces in greater detail and devise improved methods for their regeneration and repair. It is anticipated that strategies employing a combination of biomimetic stratified multi-phased or gradient-based scaffolds, advanced bioreactors capable of delivering biomimetic physico-chemical and mechanical environments, and optimal cell sources would achieve desired cellular differentiation and regeneration of musculoskeletal tissue interfaces.[49]

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