

Abstract

AN EMBEDDED BIOPRINTING PLATFORM TO GUIDE THE FUSION, PHENOTYPE AND REMODELLING OF MICROTISSUES TO ENGINEER ANISOTROPIC MUSCULOSKELETAL TISSUE

Thesis by

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Lesions to musculoskeletal tissues such as articular cartilage, meniscus, ligaments and tendons are highly prevalent and difficult to treat due to their limited intrinsic healing capacity. Current surgical strategies often yield inconsistent outcomes and rarely restore native structure, zonal organisation or mechanical function, underscoring the need for regenerative approaches that generate anatomically and biomechanically faithful grafts. Harnessing cellular self-organisation via mesenchymal stem/stromal cell (MSC)-derived microtissues (μ Ts) offers a promising route to engineer such constructs, but strategies for high-density, scaffold-free bioprinting of μ Ts into anisotropic musculoskeletal tissues remain poorly defined.

This thesis developed a microtissue-based bioprinting framework that investigates fusion, cellular self-organisation and spatially patterned biochemical cues to engineer large, structurally organised musculoskeletal grafts. First, the influence of μ T maturation and density on cartilage graft formation was investigated. Less mature (day 2) BM-MSC-derived μ Ts fused more rapidly, exhibited enhanced N-cadherin expression, and generated constructs with higher sulphated glycosaminoglycan and collagen content, while remaining low in calcium deposition. Increasing μ T density further increased matrix synthesis, and fusion of 4,000 μ Ts produced clinically relevant-sized cartilage grafts, albeit without zonal organisation. Building on this, extrusion-based bioprinting of early-stage μ Ts (F-D2) in gelatin-based bioinks into xanthan gum (XG)-derived support baths established that stable, methacrylated XG (XG-MA) baths were critical to prevent tissue shrinkage, preserve printed architecture and promote anisotropic extracellular matrix (ECM) deposition.

These insights were extended into a new 4D bioprinting platform in which MSC-derived μ Ts were patterned within support baths of tunable stiffness that acted as temporally evolving physical boundaries. Bath stiffness modulated YAP activity and directed MSC fate under uniform TGF- β 3 exposure, enabling the generation of cartilage-like (type II collagen-rich), fibrocartilage-like and ligament-like (type I collagen-rich) tissues. The tissue maturation driven by the physical boundaries (support bath) promoted collagen alignment along the printing direction, yielding anisotropic constructs. To overcome incomplete filament fusion in scaled grafts, semi-IPNs composed of XG and XG-MA were formulated to develop a more degradable support bath; a 1:1 blend preserved collagen alignment while enabling progressive bath degradation, improved tissue cohesiveness and increased transverse stiffness relative to XG-MA alone. Finally, this refined platform was applied to engineer zonally organised meniscal grafts. Dual-bioink printing with laponite-based formulations presenting TGF- β 3 in the inner zone and CTGF + TGF- β 3 in the outer zone produced large meniscal constructs that recapitulated key regional differences in ECM composition, with inner regions enriched in sGAG and type II collagen and outer regions dominated by type I collagen.

Collectively, this thesis established a versatile μ T bioprinting strategy that integrated cellular self-organisation with bioprinting to generate anisotropic, MSC-derived musculoskeletal grafts, including a zonally defined meniscus, with enhanced translational potential for patient-specific tissue repair.