Engineering functional skeletal muscle networks by microfluidic bioprinting

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In mammalians, myogenesis is a complex phenomenon starting from the very first weeks of embryonic development. This process involves mononucleated cells named myoblasts that progressively fuse forming plurinucleated syncytia named myotubes. As development proceeds, myotubes undergo a maturation process, they grow in size, and eventually the actin-myosin based contractile apparatus is assembled, together with the neuromuscular and myotendinous junctions. Skeletal muscles can self-repair relatively small damages resulting from tears, small lacerations, strains, or toxins via a three-stage process that involves demolition, repair, and remodeling of myotubes. However, skeletal muscle cannot restore significant tissue loss that can arise after severe trauma, invasive surgeries, or degenerative diseases.

3D bioprinting has the potential to fabricate highly customizable and highly organized structures that, in principle, could be used for the assembly of an entire muscle [1]. This emerging biofabrication technology relies on the simultaneous deposition of cells and biomaterials in a layer-by-layer fashion, to form 3D well-organized heterogeneous structures that can mirror relevant complex biological architectures both physiologically and morphologically. Thanks to these attractive features, 3D bioprinting is rapidly becoming a first-choice technique for a broad set of tissue engineering (TE) scenarios, including skeletal muscle tissue reconstruction [2]. Inspired by the native structural morphology of skeletal muscles, we speculated that the spatial confinement of muscle precursor cells (C2C12) into highly aligned and compact 3D bioprinted hydrogel fiber structures could lead to a better orientation of the arising myotubes, thus mimicking the natural muscle morphology and organization more closely [3,4]. Building on such a premise, we developed a 3D bioprinting strategy based on a custom microfluidic printing head coupled to a co-axial extruder (Figure 1). This system enables the high resolution deposition of multi-material and multi-cellular structures by simultaneously extruding different bioinks or by rapidly switching from one bioink to another. Within few days of in vitro culture following 3D bioprinting, C2C12 started to elongate and fuse, forming highly aligned myotubes. The obtained myo-structures were thoroughly analyzed in terms of myotube length and orientation, fluorescence immunocytochemistry, and gene expression of relevant myogenic differentiation markers (MHC, MYOD, MYOG), revealing a significant differentiation and maturation of myotubes (Figure 2). Moreover, we demonstrated in vivo that the 3D bioprinted constructs outperformed control bulk-hydrogels in terms of myotube structural organization, supporting the hypothesis that the simple geometrical confinement exerted by 3D bioprinted hydrogel fibers promotes the architectural organization of muscle precursors cells.

These studies have the potential to unveil the mechanisms by which muscle precursors sense substrate stiffness and confinement, therefore representing a key starting point for the development of novel skeletal muscle regeneration strategies.

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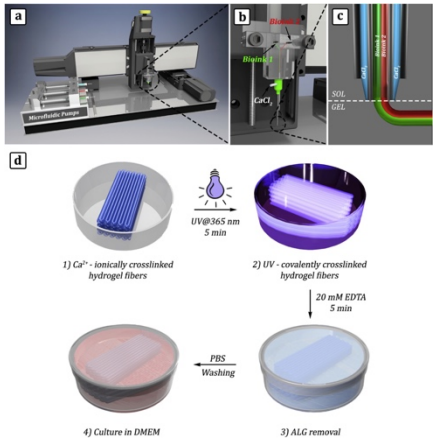

Figure 1. (Top) 3D bioprinting set-up composed of syringe pumps and a microfluidic printing head coupled to a coaxial extruder. (Bottom) Scheme of the cross-linking procedure.

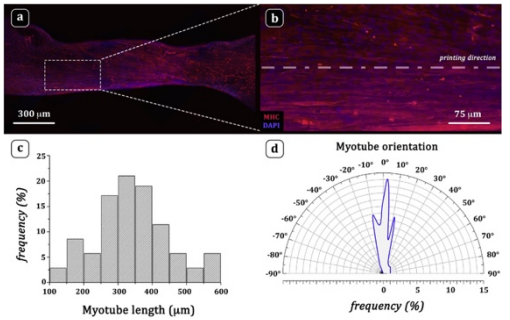

Figure 2. Quantification of myotube alignment and length for 3D bioprinted constructs after 15 days in vitro.