CTI – Start-up and Entrepreneurship, R&D Funding, KTT Support

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A novel multiwell device for drug development with bioprinted 3D human tendon and skeletal muscle tissues

Project Number: 16313.1 PFLS-LS
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Industrial Partner: regenHU, Marc Thurner, Andreas Scheidegger
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Project Goal

There is a huge medical need for treatments of degenerative muscle and tendon diseases in our aging societies. Currently, there are no approved pharmaceutical therapies. A key component of successful drug development is the availability of organotypic cell culture disease models for efficient physiological compound screening. 3D Bioprinting is a new technology for the in vitro engineering of human living tissue using a 3D printer. We intend to develop a novel multiwell tissue culture system consisting of bioprinted human skeletal muscle and tendon tissues anchored between intelligent posts that allow mechanical stimulation and functional analysis. This system may also at least partially replace animal-based ex vivo muscle and tendon assays.

Key Findings

- **Tissue engineering of muscle and tendon tissue with 3D bioprinting is feasible**
- **BioInk™ is cell-compatible with respect to viability, proliferation and differentiation (contraction of muscle tissue, collagen I distribution in tendon)**
- **Two concepts of innovative 24 well plate systems to enable muscle/tendon tissue production and analysis are developed.**

Tissue Engineering

Human skeletal muscle derived cells (SkMDC, myoblast) were printed with BioInk™ in a layer-by-layer mode in a dumbbell-shape and were let to proliferate and differentiate ~~and were differentiated~~ (Fig. 1A and B). The two holes are the placeholder for the posts.

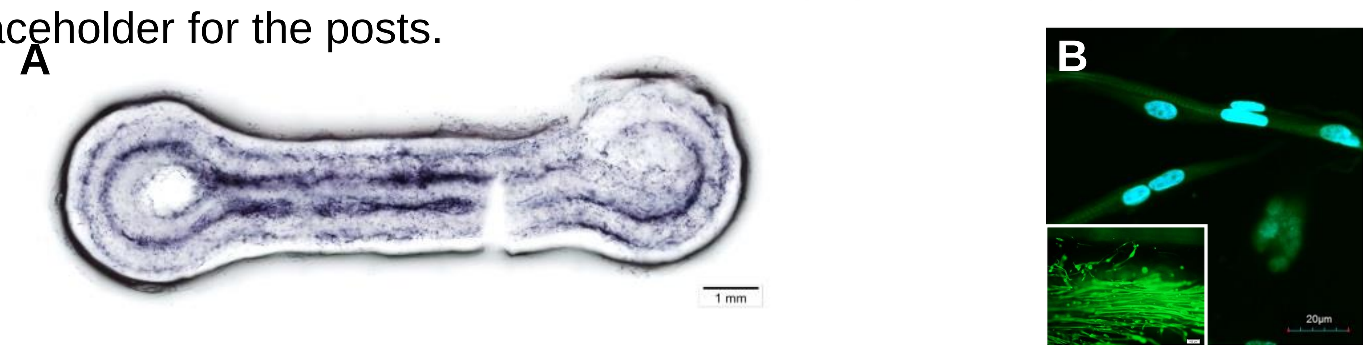

Figure 1: Myoblasts printed with BioInk™ in a layer-by-layer mode and differentiated for 7 days. A) Viability staining using MTT. Scale bar 1 mm. B) Immunostaining of myosin heavy chain in differentiated myotubes (green, in blue DAPI-stained nuclei). Insert shows bundles of myotubes. Scale bar 20 µm, Scale bar from insert 100 µm .

In the same way primary rat tenocytes were printed with BioInk™ and were differentiated (Fig. 2A and B). First co-printing approach to deposit tenocytes at the borders and myoblasts in between the posts were successful (Fig. 2C).

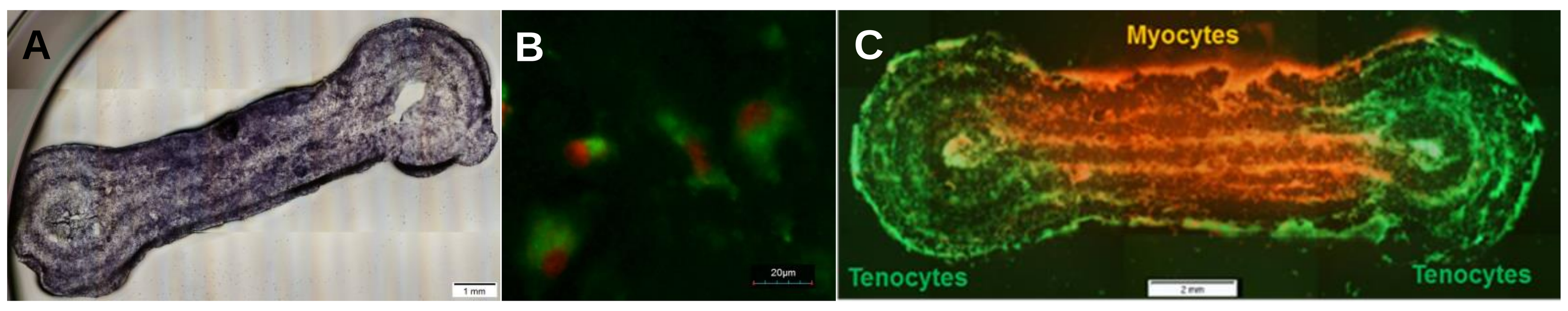

Figure 2: Primary rat tenocytes printed with BioInk™ in a layer-by-layer mode and co-culture model. In A) Viable tenocytes were stained with MTT, 11 days after printing. Scale bar 1 mm. B) Collagen I immunostaining of differentiated tenocytes for 5 days. The characteristic collagen I distribution around the nuclei is observed. (green, in red propidium iodide-stained cell nuclei). Scale bar 20 µm. In C) printed tissue model with pre-labeled myocytes (in red) and tenocytes (in green) shows the feasibility to print two different cell types at defined locations within a construct. Scale bar 2 mm.

Bioprinting

Soft- and hardware (Fig. 3) of the bioprinting device 3DDiscovery® ~~(Fig. 3)~~ were adapted to print into 24 well plates. The heights of UV-polymerization unit, inkjet and BioInk™deposition unit were adjusted to print from 2.2 cm above the well plate bottom.

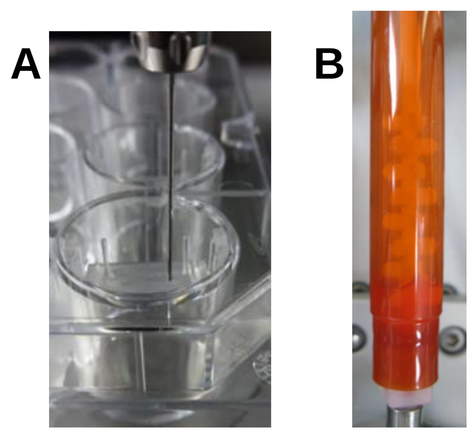

Figure 3: Hardware adaptation of the 3DDiscovery® bioprinting device. A) shows the extended needle to print into 24 well plates in contact printing mode (BioInk™ deposition). In B) the cell mixing system inside the cartridge to allow homogenous cell printing in jetting mode is shown.

Innovative multiwell device

Key for the development of in vitro muscle/tendon tissue will be novel inserts containing attachment posts. Each well will contain two posts for tissue fiber formation ~~and~~ allowing mechanical/electrical stimulation and functional readouts (Fig. 4A and B).

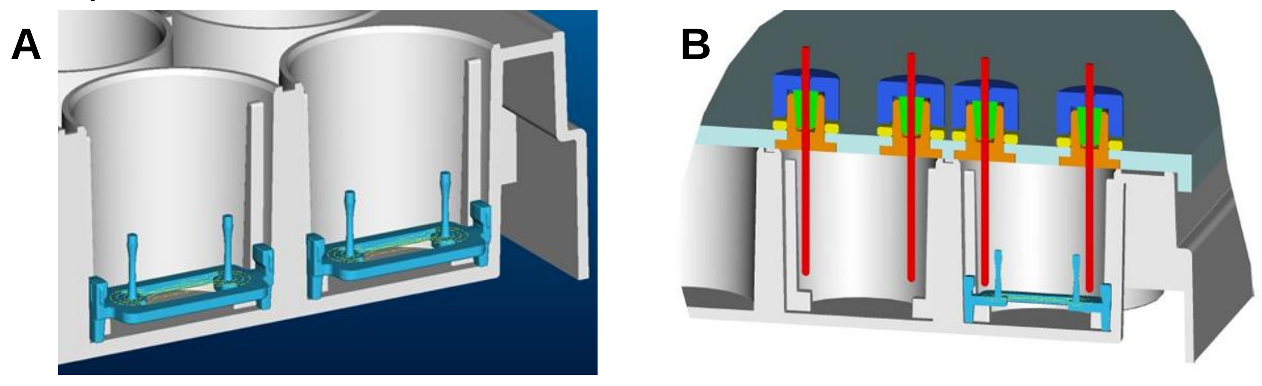

Figure 4: Postdesign for functional tissue printing. A) Shows in blue the postholder in a 24 well plate. Around the two posts ~~a~~ muscle/tendon tissues are printed as shown in Fig. 1A and 2A. The produced tissues can be observed with inverted microscopes. In B) a concept of electrode (in red) integration into the well plate is shown allowing electrical stimulation of the ~~produced~~ printed tissues..

Scientific Innovation

- **Novel 24 well plate system**
  - **Each well contains inserts with two microstructured posts for muscle/tendon tissue generation.**
  - **Prototypes include**
    => electrodes for electrical stimulation of the muscle/tendon tissue.
    => cantilever to measure muscle contraction for measure muscle contraction.
- **Routine and robust production of 3D human muscle/tendon tissues with bioprinting technology**
- **Combining a specialized 24 well plate device with bioprinting technology for complex tissue engineering and physiological readouts**

Acknowledgements

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Business Potential

- **Market growth in tissue engineering and especially in orthopedics, musculoskeletal and spine treatment will double between 2013 and 2018**
  - **Encourages to develop new effective medications and treatment methods**
  - **Need for higher automated and specific multiwell devices for drug development**
- **We develop a novel all-in-one solution for medium throughput drug development including:**
  - **Innovative well plates**
  - **Bioprinter device with application notes**
  - **Reliable muscle/tendon tissue generation**
- **Business potential is shown by the market pulls from Novartis and other companies active in this field.**
- **Weidmann Medical Technology AG and regenHU have direct access to the market regarding systems and consumables**

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