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Molecules in focus

Single muscle fibre biomechanics and biomechatronics – The challenges, the pitfalls and the future

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ABSTRACT

Interest in muscle biomechanics is growing with availabilities of patient biopsies and animal models related to muscle diseases, muscle wasting (sarcopenia, cachexia), exercise and drug effects. However, development of technologies or facilitated systems required to measure biomechanical and contractile properties of single fibres has not kept pace with this demand. Most studies use manual mechatronics systems that have not changed in decades and are confined to a few labs worldwide. Available commercial systems are expensive and limited in versatility, throughput and user-friendliness. We review major standard systems available from research labs and commercial sources, and benchmark those to our recently developed automated *MyoRobot* biomechatronics platform that provides versatility to cover multiple organ scales, is flexible in programming for active/passive muscle biomechanics using custom-made graphics user interfaces, employs *on-the-fly* data analyses and does not rely on external research microscopes. With higher throughput, this system blends *Industry 4.0* automation principles into myology.

1. Skeletal muscle biomechanics – organ-scales set technology demands

Skeletal muscle is a structurally highly organized organ. Actomyosin motorprotein filaments are arranged in long myofibrils. A few thousand myofibrils are parallel arranged within each muscle cell, or myofibre. A large number of parallel myofibres connected by extracellular matrix (ECM) compose a macroscopic muscle. Muscle performance can be either through active contraction or giving in to passive stretch. Depending on the load, contractile modes range from purely isometric (infinite load, no movement) to purely isotonic (constant/no load). Passive elasticity and viscous behaviour define muscles' capabilities to comply with external strains. Morphological organ scales (whole muscle, fibre bundles, single fibres), strongly determine the mechanical output, i.e. movement and force development, whether it involves studying a single fibre, fibre bundles or a whole muscle, due to different contributions from ECM or anchorage points (Grossberg et al., 2011).

When assessing muscle biomechanics in biomedicine, two general challenges apply, (i) one relates to muscle sample preparation and (ii) one to the metrology for force assessment. In general, the smaller the desired organ scale to study, the more cumbersome and tedious both aspects become. This holds in particular true for mechanical bioseparation of single myofibres from the whole muscle, or even special downstream processing procedures, like mechanical skinning (peeling off the sarcolemma from a fibre) (Lamb and Stephenson, 2018), all of which must be performed by hand with very low throughput per animal or day. Alternatively, chemical isolation of single myofibres (Kiriaev et al., 2018) and even myofibrils (Schürmann et al., 2010) provides much higher yields (Kiriaev et al., 2018), but fibres may not be suitable for very specialized biomechanics protocols (Lamb and Stephenson, 2018).

The technology principle for muscle biomechanics, on the other side, is fairly simple: it requires an end-to-end fixation of a muscle sample between two pins, one with a finite known stiffness acting as a force probe while the other opposes with a practically infinite stiffness. Force sensors exploit Hooke's law in their linear range, converting deflections of the sensor pin to forces by employing piezo-resistive, piezo-optical or other principles (Stefanescu, 2011). Although the concept is

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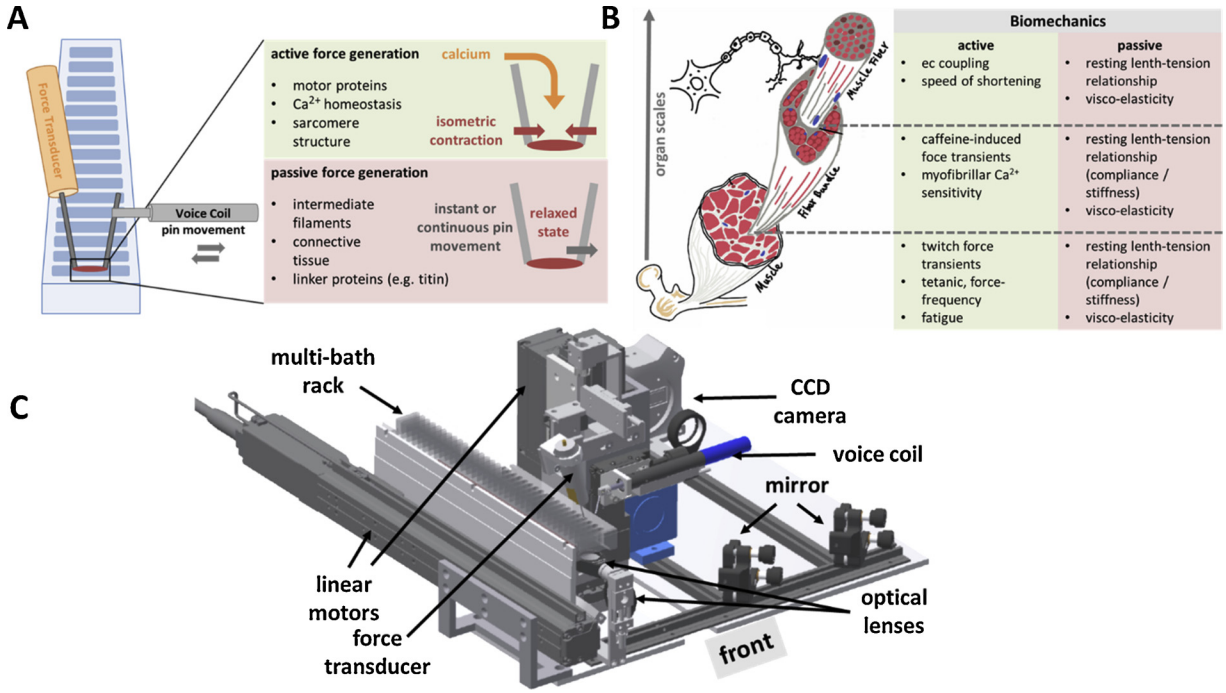


Fig. 1. Levels of biomechanics assessment across several organ scales of skeletal muscle using the automated *MyoRobot* biomechatronics platform. A, active force as well as passive visco-elasticity assessment can all be implemented using a linear force transducer-voice coil actuator configuration allowing fixed length or flexible length extensions/*slacks* with high precision in the very fast and slow actuation range. In addition, the system is versatile to cover organ scales from whole muscle down to single fibres **(B). C**, auto-CAD 3D representations of the *MyoRobot* system with in-built microscopy optics. Note that the front ridge contains an in-built sled to slide in and out a simple dissecting binocular for fibre mounting not shown here but elsewhere (Haug et al., 2019).

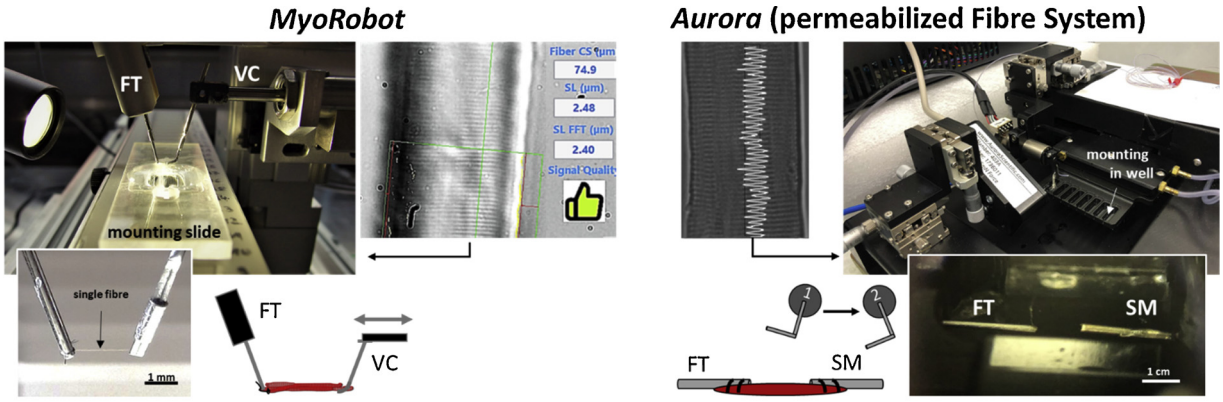
universal, the smaller the preparation scale (*e.g.* single fibres over whole muscle), the more miniaturized and thus, challenging the mounting and operation procedures become. In general, what all biomechanical attempts have in common is the fixed mechanical connection between the samples and the recording apparatus. This is still performed manually in current studies therefore, requiring elaborate skills of single cell micro-dissection and micromanipulation (Lamb and Stephenson, 2018; Roche et al., 2015). Apart from few studies on whole muscle assessing lateral force transmission (Ramaswamy et al., 2011; Boriek et al., 2001), the focus of biomechanics studies is on axial active/passive properties. Single fibres represent a very pure muscle preparation however, due to their delicate nature, the range of available technologies for their assessment is rather limited, as are studies involving single fibres (see below).

Most interventions on single fibre biomechanics require to permeabilize and expose fibres to chemically defined environments simulating various activating or relaxing conditions *in vivo*. With such chemical manipulations, one can apply defined contractile activation or relaxation procedures on fibres to assess their passive visco-elastic properties. This can be performed by milli-/micro-fluidics systems for solution exchanges (Lin et al., 2001). However, more commonly, the whole 'transducer-preparation-counter pin' block is actuated to plunge into subsequent bathing chambers for defined times, to either relax, activate or Ca^{2+}-load biocompartments within a fibre (Lamb and Stephenson, 2018; Moss, 1979). The single fibre preparation is manually transferred through the liquid-air phase twice, while the solution bath is manually exchanged underneath the preparation. This classical methodology goes back as early as 1969 (Hellam and Podolsky, 1969; Ashley and Moisescu, 1977) and is still basically used in its original configuration today (Lamb and Stephenson, 2018). The use of this somewhat archaic set-up means that stopwatches are still used to time the incubation of fibres in drugs or activating solutions introducing a level of variability that is no longer acceptable in most areas of muscle research today. In particular, the requirement to precisely monitor incubation times of muscle fibres in chemical solutions to the second, to manually manoeuver baths to the desired well, to adjust fibre lengths using manual micro-screws, up to data documentation on paper charts or digitized chart recorders with subsequent manual data analysis; all those have prevented the methodology to be disseminated to many labs worldwide. Thus, single muscle fibre biomechanics research has been restricted to or been contained within a handful of labs (Head, 2010; Lamboley et al., 2015; Hvid et al., 2011; Haug et al., 2018; Bottinelli et al., 1994; Brocca et al., 2017; Reid et al., 2002; Lamb and Stephenson, 2018; Slivka et al., 2008), also simply because of the lack of automation for repetitive mechanical and electro-optical sequences. Moreover, larger throughput pharmacological single fibre biomechanics testing is not possible this way.

The demand for biomechanical assessment of single muscle fibres increases due to increased availabilities of genetically-modified mouse models or patient biopsies, as reflected by metrics for muscle biomechanics studies (PubMed results for *skeletal muscle force*, yields about 1000 publications per year; last 10 years). However, numbers of single fibre biomechanics studies not only stagnate but account for less than only 0.1% (PubMed results for *skeletal muscle force single (fiber OR fibre)* yield around 20 papers per year). Clearly, single fibre biomechanics would vastly benefit from automation procedures, mostly regarding speed, precision and reproducibility of data acquisition. This would also open new venues for clinical testing. Although the manual and delicate single fibre bioseparation and preparation processes are hard to automate and still remain the domain of human researchers, those could be facilitated as well by use of standardized enzymatic bioseparation procedures for most biomechanical assays.

2. Single muscle fibre biomechatronics – not really a question of commercial choice

Some labs have been sticking to the originally designed manual biomechatronics systems technology for decades, with minor or no modifications regarding automation (Lamb and Stephenson, 2018). When overseeing the global market for biomechatronics vendors in the



• pins: angular orientation, facing downwards. Mounting: one end tied with knot while submerge, other VC-pin clamp-tweezer. Easy mounting in wide mounting slide before lowering fibre into bath. Perpendicular fixation is less prone to fibre slipping.	• pins: parallel horizontal orientation. Slip ties over pins with wide loop, then slide end of single fibre through slipknot before closing. Fibre may end up on opposing sides of pins introducing unknown torque. Tedious mounting within tiny chamber requires intensive training. Fibre slipping may occur.
• after mounting, no manual alignment necessary.	• after mounting, pins are manually aligned parallel with micrometer screws.
• VC performs steady, slow (resting length-tension curves) or very fast (slack-tests) precision movements (current feedback).	• Stepper motors produce abrupt 'stop-and-go' movements. In addition, lever arm configuration can induce rotational artifacts (above ①→②).
• >20 wells → serial execution of multiple active/passive testing regimes in same fibre; suitable for pharmacology. More pCa steps available. Single well volume temperature increase: 5 min/1 °C (at T >30 °C). Glass bottom UV glued, no leakage.	• only eight bath chambers; limited capabilities for serial execution of multiple protocols on same fibre; pCa-force accuracy may become limited. Small well volume enables fast temperature jump experiments; glass bottom sealed with vacuum grease, leakage can commonly occur.
• in-built optics, no need for external research microscope; continuous imaging of the fibre. In-built automated image processing routines: multiple strategies to extract fibre diameter and sarcomere lengths (SL). Assumes circular fibre cross-section. Ca^{2+} imaging not implemented. Fluorescence imaging thinkable.	• requires external inverted (fluorescence) microscope and long working distance objectives to image fibre. A prism in one well allows side view of fibre to reconstruct elliptical fibre-cross section. No automated communication between optics and biomechatronics system. Add-on hardware systems (900/901B) provide image analysis algorithms. SL via laser diffraction (laser safety!) does not account for fibre inhomogeneities.

Fig. 2. Comparative benchmark analysis of the MyoRobot versus the Aurora permeabilized fibre system. Shown are images of both systems showing the fibre mounting in a vertical (*MyoRobot*) and horizontal (*Aurora*) configuration, respectively. The single fibre image shown for the *MyoRobot* was recorded and on-the-fly analysed for diameter and sarcomere length using the in-built optics while the fibre for the *Aurora* system was captured using a camera attached to the microscope (HVSL 901B). The table summarizes eminent characteristics and differences of both systems. FT: force transducer. VC: voice coil actuator. SM: stepper motor.

field of muscle biomechanics, only *World Precision Instruments* (WPI, https://www.wpiinc.com/) and *Aurora Scientific* (https://aurorascientific.com/) provide ready-to-use systems. WPI recently introduced their '*Cell Tester*' system (https://www.wpiinc.com/si-cts200-cell-tester-200), a prototype of which has been used in one study assessing single fibre force so far (Khairallah et al., 2012). That system incorporates WPI force transducers and length controller stepper motors mounted on micromanipulators, but does not include any internal optical assessment or readout components. It fully relies on the availability of an existing inverted research microscope and other length assessment instrumentation, like the *Aurora Scientific* high-speed video sarcomere length camera in Khairallah et al. (2012). *Aurora Scientific* has certainly been the market leader for many years in distributing biomechatronics systems for muscle research. Many muscle biomechanics labs have used or are still using one of their systems (Roche et al., 2015). Probably as part of their business model, systems covering different preparation scales, *e.g.* whole muscle or single myofibres, always come as separate system packages. Like the *Cell Tester*, they rely on external optical readout systems, *e.g.* inverted microscope, and additional length assessment hardware (*e.g.* 900/901B HVSL system) to purchase separately thus, uncoupling biomechanics from optics within the system. The optical performance of the separate microscope allows to combine fibre geometry with, *e.g.* fluorescence recordings. A prism in one well also allows two-plane imaging and elliptical fibre geometry reconstruction. A complete '*Permeabilized Fiber System*' by *Aurora Scientific* (https://aurorascientific.com/products/muscle-physiology/systems/permeabilized-fiber-microscope/) can readily exceed 50 kUSD in costs which also explains its relatively exclusive scientific distribution (Ochala and Larsson, 2008; Wood et al., 2014; Roche et al., 2015). Similar to manual systems (Lamb and Stephenson, 2018), it also features movable baths within a rack. However, due to design constraints given by microscope stages and related stability considerations, only eight miniature baths are present, and mounting of a fibre must be performed under narrow space conditions, manually tying a fibre with multiple micro-knots. The length controller consists of a fast stepper motor for step changes within 200 µs of ~1 µm resolution. Although the system covers most functionality on the single fibre level, the hardware and software development in terms of automation and flexibility across organ scales has not been pursued within many years. In view of the aforementioned demand for more single fibre studies, the field would clearly benefit from a more facilitated, robotized and intuitive system. However, currently, there is no such choice on the market.

In an attempt to address limitations of existing systems and to increase flexibility, convenience and throughput in single fibre biomechanics, we engineered a novel automated biomechatronics platform, the *MyoRobot*, in a joint university-industry R&D project (FAU team). The system (Fig. 1) contains several engineered improvements: (1) a linear rack with a much larger number of chemical baths (currently 25, expandable) to allow for serially executed sequences of active and passive biomechanics recordings in the same single muscle fibre, also covering control-drug-washout scenarios. (2) The system also includes an innovative solution to the abrupt movements usually introduced by stepper motors in other systems. Here, high precision voice coil (VC) technology was engineered into the length controller (Haug et al., 2018, 2019). VCs are operated on Lorentz-force induction and can be very precisely and continuously driven using continuous current feedback control to the coil, thus allowing speeds between 0.4 µm/s and 250 mm/s (1 µm encoder precision). Therefore, both ends of the spectra

for steady-state elasticity and unloaded speed of shortening (*slack-tests*, Marx et al., 2006) assessment have become possible in one system (Haug et al., 2018). (3) State-of-the art "robo cylinder" linear pulse actuators with a displacement speed of 40 cm/s for payloads < 1 kg provide extremely fast bath exchanges over conventional stepper motors and minimize fibre exposure to the air interface to < 1 s, even for end-to-end bath manoeuvres (˜25 cm). One of the most notable achievements in our *MyoRobot* is (4) the recent inclusion of an optically engineered infinity optics with a ×20 magnification to allow for continuous acquisition of fibre diameter and sarcomere length (SL) and (5) on-the-fly image analysis and fibre morphometry without the need for any external research microscope. The biomechatronics and optical sensor information were (6) all combined into one LabView GUI systems control and readout interface, thus the system unifies opto-mechatronics and closes an important systems communication gap in current commercial systems. (7) Lastly, the *MyoRobot* covers recordings from several organ scales, from single fibres to whole muscle, by just exchanging transducer head and bath racks. Taking advantage of a back-to-back benchmark analysis, we compared the *MyoRobot* and the *Aurora* system for obvious differences in performance and management, handling, advantages and constraints with a few items listed in Fig. 2. The pure hardware costs related to optical, mechatronics and sensor technology components range between roughly 25–30 kUSD.

3. Conclusions

Single muscle fibre biomechanics receives growing attention to assess muscle performance in many diseases, ageing and pharmacology studies reflecting a pure one-cell preparation. However, being tedious and manually demanding, its potential has not been advanced by '*Industry* 4.0' automation and robotics incentives. Also, existing market leaders have not filled in this gap. The *MyoRobot* platform is proposed to increase the overall availability of muscle biomechanics assessment in basic muscle research, but more importantly, in biomedicine and clinical diagnostics. A market introduction for this system is currently prepared and could also include expanding the ease-of-use to the end user by introducing ready-to-go internal solution kits (e.g. for pCa-force assessments). Current details on the *MyoRobot* system can be found at https://www.mbt.tf.fau.de/research/research-groups/muscle-biomechatronics/the-myorobot-prototype/.

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