Bioreactor system to apply shear stresses to stem cells *in vitro* for studying mechanobiology

Scope: Control and evaluate a low-cost bioreactor system to apply user-defined magnitudes of shear stress to mammalian cells in culture to explore scientific questions in mechanobiology.

Background: Cells embedded in tendon experience shear stresses as the collagen fibers slide past each other during mechanical stimulation (**Figure 1A**). These shear stresses have the potential to impact the biological response of the tendon cells. Investigating how shear stresses regulate cell behavior are needed to better understand the mechanobiology of tendon. Dr. Nathan Schiele's (Chemical & Biological Engineering, University of Idaho) tendon tissue engineering laboratory is interested in studying how shear stress impacts the differentiation of stem cells toward to tendon lineage (tenogenesis) and tendon formation. To conduct these studies, a bioreactor system has been designed that can apply fluid shear stress to cells in culture (**Figure 1B,C**). While this system appears to be effective, there is a need to refine and validate the design. Specifically, the control system can be reduced to a single microcontroller and integrated into a web interface (web-ui). Further, the device needs to be validated for *in vitro* cell culture.

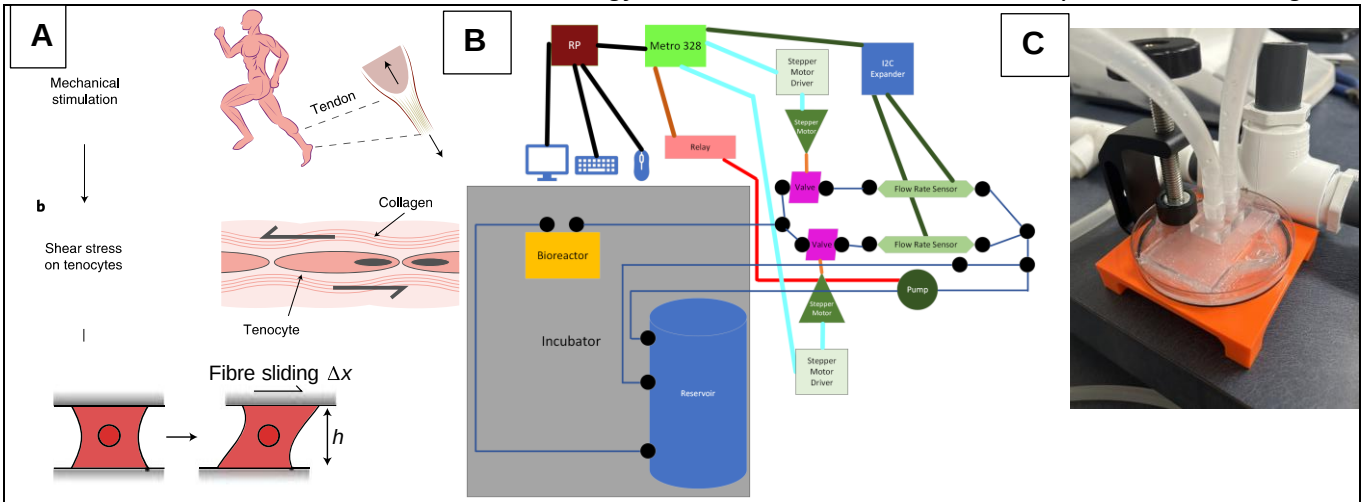

Figure 1. A) Cells in tendon experience shear stress as collagen fibers slide past each other during tendon stretching (Passini et al., 2021). **B)** Schematic for the shear stress bioreactor system. **C)** Culture chamber where fluid shear stress is applied to cells.

Specific Design Requirements:

- Fit inside a standard cell culture incubator (~14" W, 12" H, 12" L)
- Maintain and withstand incubator conditions needed for mammalian cell culture (37°C, CO_2 at 5%, and ~95% relative humidity)
- Control shear stress applied to the cells with magnitudes from 0 to 150 mPa (Maeda et al., 2011) for longer term (days to weeks), or 2 Pa for shorter term (seconds to hours) experiments
- Apply constant or cyclic shear stress (frequency range from 0 to 2 Hz) with shear stress application times of 0 to 24 hrs/day
- Maintain stimuli and culture conditions for up to 21 days
- Cell culture usage should be kept to a minimum to reduce cost of experiments (for example a typical 6-well has a working volume of 2 mL and T75 culture flask has volume of 10 mL)
- Components placed inside the incubator must be able to be sterilized (autoclave or 70% Ethanol)
- User friendly web interface that is controlled via a single microcontroller
- Low-cost to be easily replicated across different labs (maximum budget of $1500)

Reference:
Passini FS e al., Shear-stress sensing by PIEZO1 regulates tendon stiffness in rodents and influences jumping performance in humans. Nat Biomed Eng. 2021 May 24. doi: 10.1038/s41551-021-00716-x. Epub ahead of print. PMID: 34031557.