Objective 2: System validation – verification of dosage delivery (reviewer response)

Pre-calibrated pump performance under simulated *in vivo* conditions provides a suitable means for validating delivery. The following methodology will be implemented to validate pump performance. For all experiments, averages and standard errors are calculated and statistical differences evaluated by student t-test and ANOVA ($p < 0.05$).

Pumping at different currents will be performed under both wired and wireless conditions. Precise timing of activation will be controlled through the software user-interface. Flow rate is calculated from measurements of pumped volume through a calibrated micropipette; a 2 µL air bubble is introduced into the pipette and tracked with a stereomicroscope and micrometer. The influence of catheter diameter (1.2-3 Fr) and length (1, 2, 4, 8 cm) will be determined. Operation under backpressure (11 mmHg is the venous pressure of the mouse) will be evaluated (5-20 mmHg). For each flow experiment, a minimum of 6 pumps will be used and each in at least four trials for each test condition.

Simulated biological materials will be used to demonstrate wireless operation and investigate the impact of electrical field distribution in the body on wireless pump operation. Simulated tissue consisting of hydroxyethyl cellulose-based gels will be prepared according to established protocols[89]. For each experiment, the outflow catheter will be embedded in a fresh gel block or the outflow collected and weighed on a precision analytical balance to confirm that the desired dose volume was delivered.

3 PHASE I RESEARCH PLAN

The research plan consists of 3 objectives with multiple stages shown in Figure 10 and described in detail below. The primary goal of this Phase I proposal is to demonstrate the feasibility of the electrolysis-based Fluid Sync micropump. At the conclusion of Phase 1, the pump system prototype will be demonstrated at the benchtop and benchmarked for its precision and accuracy.

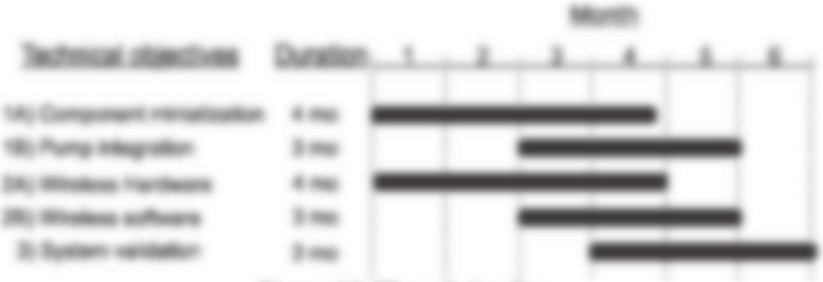

Figure 10 Phase 1 timeline.

3.1 Objective 1A: Miniaturize pump components

Electrodes and Nafion Coating: Electrolysis electrodes will be fabricated on soda lime glass substrates using established techniques for the lift-off patterning of thin film metals[85]. Electrodes will be potentiostatically cleaned at 0.5V in 1X phosphate buffered saline. Nafion will be coated onto electrodes to prevent bubble occlusion of the active electrode surface and achieve higher efficiency[86, 87]. Coating will be performed after electrode fabrication by dip coating (2× for ~ 1µm coating) according to Jones et al.[86] using < 9 wt% Nafion for optimal performance. Evaluation of electrodes will entail electrochemical impedance spectroscopy to characterize electrode-electrolyte parameters (double layer capacitance, polarization resistance, and electrolyte resistance) and measurement of Nafion coating thickness.

Bellows: Circular multi-convolution bellows will be designed to match the electrode layout and measure no more than 10 mm in diameter and 5 mm in height to accommodate the size constraints for use in mice while at the same time minimizing any dead volume. Mechanical design and simulation of the bellows