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Development of a current controlled stimulation setup for investigating the effect of electrical currents on implant infections caused by biofilms

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Abstract: **Introduction:** Biofilms are bacterial communities that transform into a state enclosed in an extracellular polymeric substance, which makes them less susceptible to antibiotics. Those bacterial formations often develop on metal implants and cause chronic infections. Due to the severely reduced impact of antibiotics against biofilms, the effect of electric stimulation (on its own and in combination with antimicrobials) needs to be further observed, as available studies indicate a positive effect. **Methods:** Therefore, this work examined the development of a six-channel current-controlled stimulation setup, which enables further in vitro research on the effects of electric stimulation on biofilms. The setup controls the desired stimulation current through the load to counteract electrochemical processes, which constantly change its resistive and capacitive properties. **Results:** Each channel of the stimulator is able to operate within an amplitude range of $50\ \mu\text{A}$ to $1\ \text{mA}$, a frequency range of $0\ \text{Hz}$ to $1\ \text{kHz}$, and a pulse width range of $50\ \mu\text{s}$ to $1\ \text{ms}$. The current control provides a sufficient rise time of $3.3\ \mu\text{s}$ for three different stimulation modes: constant direct current (DC), pulsed DC, and biphasic-pulsed alternating current (AC). Furthermore, a graphical user interface enables the user to regulate and observe the stimulation on a computer to which the stimulator device is connected. **Conclusion:** The achieved variety of stimulation parameters in one device makes it possible to analyze the effect of different stimulation paradigms on biofilms and therefore enables more in vitro research which is inevitable to develop a sufficient treatment for patients with biofilm-infected implants.

Keywords: electric stimulation (ES); biofilm; implants

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1 Introduction

Biofilms are bacterial structures that develop on biotic and abiotic surfaces, often cause chronic infections, and have a 500-5000-fold increase in resistance against antibiotics when comparing them to bacteria in their planktonic state [1]. Treating biofilms with an increased dose of antibiotic cannot only drastically increase its harmful side effects but also the risk of developing a multi-drug resistant strain [2]. Consequently, alternative treatments are needed. Several studies have shown the effect of different kinds of electrical stimulation on its own and together with antimicrobials on the biofilm (e.g., [2, 3]). Even though the exact way the electrical current decreases the biofilm is not clear, there are many theories. It was observed that high voltage directly damages the cell membrane and low voltages/currents seem to affect the biofilm indirectly through electrolysis [3]. Since the in vivo implementation of a high voltage stimulator comes with obvious complications, the setup presented here focuses on low current/voltage stimulation. Although a moderate amount of electrolysis is needed in order to decrease the biofilm, it is not only harmful to bacteria but also to human cells. The extent of electrolysis depends on the amount of current passing through the electrodes and therefore the focus was to build a low current/low voltage current-controlled stimulation setup. The setup provides a significant variety of stimulation signals which enables the research to test the effect of as many different current amplitudes and stimulation modes as possible. An innovation is, that those stimulation signals are individually current-controlled for six loads (Petri dish compartments) while existing solutions use only one controlled current for stimulating multiple loads in parallel under the unrealistic assumption of equal loads in all compartments [4].

2 Methods

The stimulator was designed for a standard six-well Petri dish. Each of the six Petri dish compartments forms a separate load, which consists of a pair of stainless steel electrodes submerged in 0.9% isotone saline solution with glass beads that contain the biofilm (see Figure 1). The freely moving glass beads and the electrochemical processes change the load's capacitive and resistive properties during stimulation. Therefore, the setup contains control circuits, which regulate the desired stimulation current through the load and counteract its nonlinear capacitance and resistance changes.

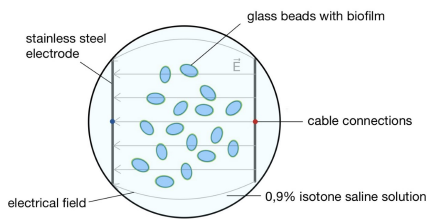


Fig. 1: Illustration of one stimulated load in a Petri dish.

As one can see in the stimulator block diagram (see Figure 2), which visualizes the main electronic structure of the device, each Petri dish load is connected to a separate control circuit. In contrast to a related study [4] where a single control circuit is regulating the current through six Petri dish compartments in parallel, the implemented control provides the benefit that the capacitive or resistive changes of a single load are addressed individually and therefore the desired stimulation in the six loads can be ensured.

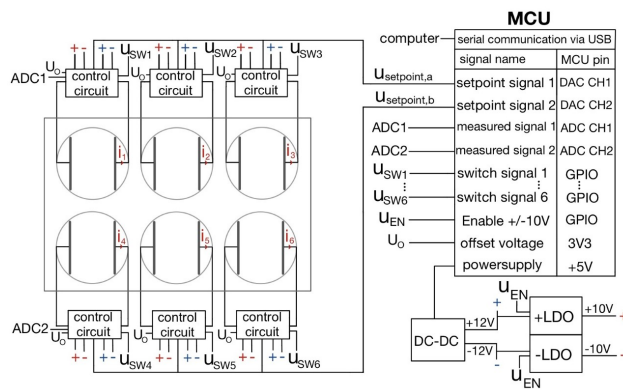


Fig. 2: Stimulator block diagram.

In addition to that, two different setpoint voltage groups ($u_{\text{setpoint,a}}$ and $u_{\text{setpoint,b}}$) generated by digital-to-analog con-

version (DAC) on a microcontroller unit (MCU) enable simultaneous stimulation with different signals. That way the electrode pairs from group a (upper three wells) can be stimulated with a different amplitude or mode than the ones from group b (lower three wells). One control circuit of each group is connected to an analog-to-digital converter (ADC) channel of the MCU. This enables the user of the stimulation setup to review the measured current through the load and thereby to check whether the desired signal was provided over the course of the entire stimulation time. Besides that, the switch signal (u_{sw}) enables the user to turn off individual control circuits while the others are stimulating. By not stimulating one of the loads a control group can be created which shows how the biofilm develops without electric stimulation over the course of the stimulation time. The stimulation setup is powered via USB from a computer over the MCU (NUCLEO-L432KC, STMicroelectronics). This link serves further for parametrization and monitoring. The electronics and stainless-steel electrodes are mounted on a 3D printed cover that fits on a standard six-well Petri dish and positions the electrodes inside the Petri dish's conductive solution. A second cover protects the electronics. Figure 3 shows the stimulation device.

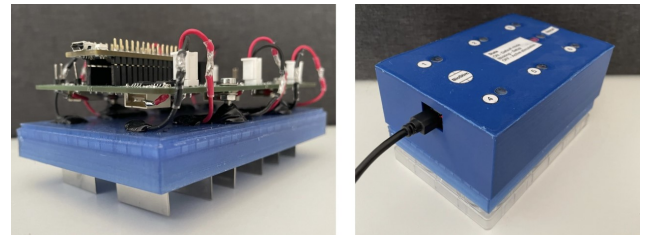


Fig. 3: Stimulation device. Left: Electronics and electrodes mounted to the 3D printed cover of the six-well Petri dish. Right: Device under operation with top cover.

2.1 Controller Design

The control circuit which was depicted as a black box in Figure 2 is shown explicitly in Figure 4. As visualized in Figure 4, the analog control circuit is composed of four main components: an operational amplifier (op amp) subtractor circuit, a low-pass filter, a voltage-controlled current source (VCCS), and a PMOS switch subcircuit. For understanding the functionality of the different components, the main controlling mechanism needs to be evaluated.

The VCCS (blue box in Figure 4) is a controller configuration where an op amp is controlling the current through a load located in its feedback loop, proportional to the setpoint voltage it receives at its noninverting input pin. The VCCS is

a well-known circuit that was also discussed in the application paper of Apex Microtechnology [5]. In this control mechanism, the stimulation current is provided by the op amp itself, which is only feasible as the device only needs to stimulate with low currents.

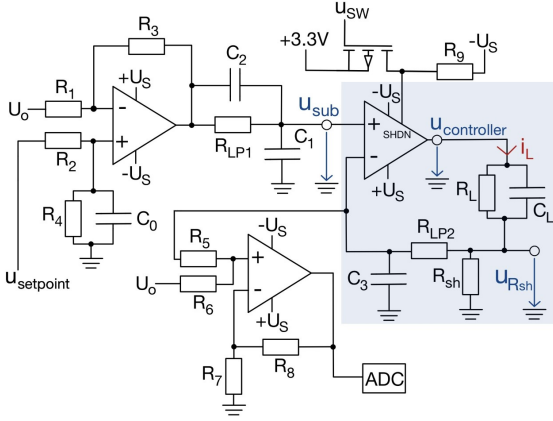


Fig. 4: Control circuit schematic, including the subtractor, the switch, the low-pass, and the VCCS subcircuit.

According to its basic simplified functionality, an operational amplifier changes the output voltage in such a manner that the input voltage difference (between inverting and noninverting input) stays as close to zero as possible. In the VCCS configuration, the inverting input voltage is equivalent to the shunt voltage $u_{R_{sh}}$, which means that the op amp controller is comparing the setpoint voltage it receives from the subtractor (u_{sub}) to $u_{R_{sh}}$ and is simultaneously trying to adjust $u_{R_{sh}}$ with its output voltage so that the $u_{sub} - u_{R_{sh}}$ is as close to zero as possible. As op amps have nearly no current flow into their input pins, almost all current that is flowing through the load i_L is also passing through the shunt resistor R_{sh} and is thereby creating the voltage drop $u_{R_{sh}}$ over the shunt resistor. So by controlling the $u_{R_{sh}}$ voltage with its output voltage to be as close to the setpoint voltage u_{sub} as possible, the operational amplifier simultaneously controls the current through the load (because of the relation $u_{R_{sh}} = R_{sh} \cdot i_L$). That way, the steady state current of the controller at a certain setpoint voltage can be determined by the following equation:

$$i_L = \frac{u_{sub}}{R_{sh}}. \quad (1)$$

According to the previously evaluated functionality of the VCCS, it needs to receive a negative setpoint voltage at its noninverting input when a biphasic-pulsed stimulation through the load should be provided. In this case, $u_{setpoint}$ needs to be shifted down, as the MCU's DACs can only provide positive analog setpoint voltages. This offset is provided by the subtractor subcircuit. After $u_{setpoint}$ received an offset from the

subtractor, the low-pass filter reduces high-frequency noise from the signal before passing it to the controller's noninverting input.

Each controller op amp is required to have a shutdown functionality, that enables the user to turn off the stimulation for an individual load, while the other controllers keep stimulating. When the MCU is applying 0 V at u_{sw} the PMOS of the switch subcircuit starts conducting, the shutdown pin of the op amp is pulled to 3.3 V, and the controller op amp is turned off (no current passes through the load). While the MCU is providing a u_{sw} of 3.2 V the PMOS is not conducting, the shutdown pin is pulled to $-U_S$, and the op amp is turned on (load receives stimulation). In order to monitor the current passing through the load, the ADC measures $u_{R_{sh}}$, which is subsequently used to calculate i_L using the previously stated Equation (1). Due to the MCU ADC's operation range being between 0 V and 3.2 V, the offset that was previously subtracted to enable biphasic-pulsed stimulation needs to be added to $u_{R_{sh}}$ before it reaches the ADC. Therefore, a noninverting op amp-adder circuit, which is adding the subtractors offset, is connected to $u_{R_{sh}}$ in the feedback loop. Preventing negative voltage from reaching the ADC is necessary because it could make the reading unusable and lead to controller instability. This could result from the interaction of the ADC and the feedback loop.

2.2 Software Design

The software design can be divided into two main categories: MCU and the graphical user interface (GUI). On the one hand side, the MCU was programmed to locally control all the electronics in the desired way. This includes providing the analog setpoint voltage to each group of control circuits, enabling/disabling the op amp's power supply (the low-dropout regulators), enabling/disabling individual control circuits by providing the switch voltage, and last but not least, reading values from the two ADC inputs (see Figure 2). In order to allow the user to interact with the electronics, a GUI was designed in pyqt. This enables the user to select the desired stimulation mode, amplitude, pulse width, frequency, and stimulation duration and to start/stop the stimulation. All the parameters that the user has selected are sent in form of an activation code via serial communication to the MCU which decodes the received data and regulates the previously mentioned stimulation signals to all the electronics accordingly. After receiving the activation code, the MCU starts to continuously send ADC measurements of $u_{R_{sh}}$ to the computer via UART, which are displayed and saved by the GUI during the stimulation to provide the user with feedback. In Figure 5 the final GUI is shown in active mode.

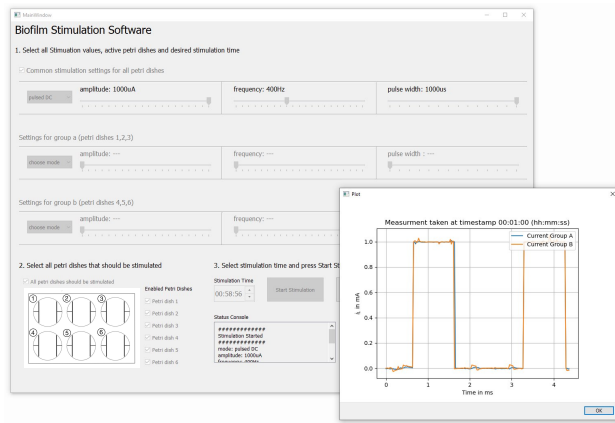


Fig. 5: Graphical user interface during a pulsed DC stimulation with an amplitude of 1 mA, a frequency of 400 Hz, and a pulse width of 1 ms.

3 Results

For the selection of electronic components, the maximum resistance and the capacity of the load (0.9% isotone saline solution and a pair of stainless steel electrodes, each 5 cm^2) have been determined to be $R_{L,\max} = 3.5 \text{ k}\Omega$ and $C_{L,\max} = 1 \text{ mF}$. The maximum necessary voltage to drive the current was estimated as $\pm 5 \text{ V}$. By powering the controller op amps with $U_S = \pm 10 \text{ V}$, the controllers are provided with a sufficient voltage reserve. The stimulation was experimentally tested (see Figure 6) and a rise time of $3.3 \mu\text{s}$ could be observed. The obtained steady-state error was zero.

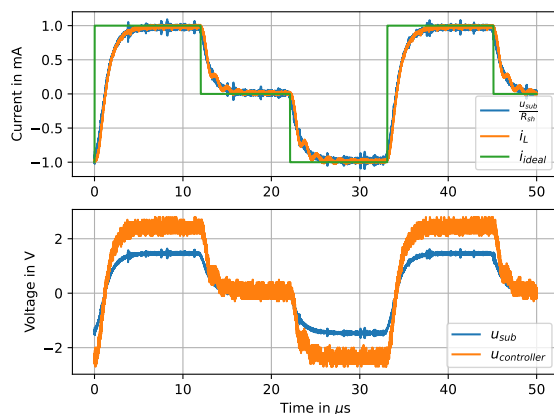


Fig. 6: Response of the current control to a biphasic setpoint voltage with pulse widths in the μs -range.

The six-channel current-controlled stimulation setup enables a variegated current simulation in a six-well Petri dish,

where the current through each Petri dish compartment is controlled individually. Besides being able to provide three different stimulation modes, DC, pulsed DC, and biphasic-pulsed AC, the control circuit is fast enough to provide pulse widths down to $50 \mu\text{s}$ and a stimulation frequency of up to 1 kHz and precise enough to provide an amplitude resolution of 20 steps between $50 \mu\text{A}$ and 1 mA .

4 Discussion

The achieved variety of stimulation parameters in one device makes it possible to analyze the effect of different stimulation paradigms on biofilms and therefore enables more in vitro research which is inevitable to develop a sufficient treatment for patients with biofilm-infected implants. The designed software additionally provides the possibility for a user to easily regulate and monitor the stimulation. In the next step, the stimulation device will be employed in studies at the Charité Universitätsmedizin Berlin. Before using the device in daily routine, the load assumption has to be verified over longer periods of time when glass beads with biofilm are inserted into the solutions.

Author Statement

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