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UREPLACE: DEVELOPMENT OF A BIOREACTOR FOR IN VITRO CULTURING OF CELL SEEDED TUBULAR VESSELS ON COLLAGEN SCAFFOLDS

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Abstract: The demand of replacements for inoperable organs exceeds the amount of available organ transplants. Therefore, tissue engineering developed as a multidisciplinary field of research for autologous in-vitro organs. Such three dimensional tissue constructs request the application of a bioreactor. The UREPLACE bioreactor is used to grow cells on tubular collagen scaffolds OPTIMAIX Sponge 1 with a maximal length of 7 cm, in order to culture in vitro an adequate ureter replacement. With a rotating unit, (urothelial) cells can be placed homogeneously on the inner scaffold surface. Furthermore, a stimulation is combined with this bioreactor resulting in an orientation of muscle cells. These culturing methods request a precise control of several parameters and actuators. A combination of a LabBox and the suitable software LabVision is used to set and conduct parameters like rotation angles, velocities, pressures and other important cell culture values. The bioreactor was tested waterproof successfully. Furthermore, the temperature controlling was adjusted to 37 °C and the CO_2 - concentration regulated to 5 %. Additionally, the pH step responses of several substances showed a perfect functioning of the designed flow chamber. All used software was tested and remained stable for several days.

Introduction: The ureter is a retroperitoneal located tube like organ with an approx. length of 25 - 30 cm connecting the renal pelvis and the urinary bladder.[1-2] A lumen with 5 – 7 wrinkles allows a stretching and an enlargement of the inner diameter, so that the urine bolus can pass.[1-2]

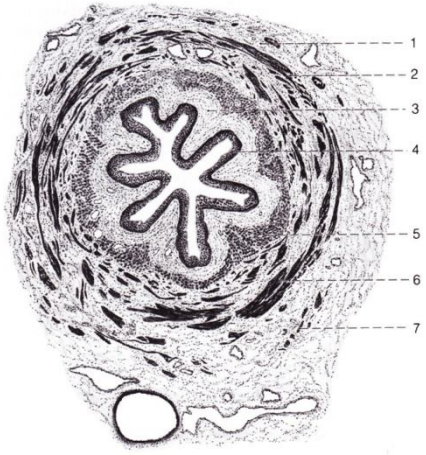

Figure 7: Cross section of the human ureter[2]; 1: adventitia; 2: smooth muscle layer; 3: lamina propia; 4: urothelium; 5: inner, mainly longitudinal directed and 6: outer, mainly circular oriented muscle fibers; 7: longitudinal oriented muscle fibers of the adventitia.

The main structural setup with its inner urothelium, 5 – 7 cell layers thick, has an inner diameter of about 5 mm preventing leakage. It is supported by the tunica muscularis containing circular and longitudinal arranged smooth muscle cells providing peristaltic movements.[1-3] The smooth muscle cells produce the necessary force in a certain frequency for the urine transport and it inhibits a reflux into the kidney.[1-3] This directed way of urine forwarding is a result of the coordinated contraction of the specifically arranged smooth muscle cells, which react on the mechanical stretching of the ureter by the urine bolus. The group of Rasidovic et al. (2010) showed in mammalian animals, that contraction frequencies and intraluminal pressures are dependent on a baseline pressure.[4]

Several diseases and malformations during development affect these functions, resulting in most cases to inflammatory reactions and the loss of the ureter.[4] Regenerative medicine tries to produce an equivalent to the physiological tissue. Therefore, tissue engineering is a suitable technology that copes with problems and challenges in three dimensional for the optimal support and arrangement of cell cultures. Such culturing methods provide great advantages in cell signaling,

spreading, proliferation and lots more, in comparison to two dimensional cell cultures. Several dynamic cultivation approaches showed a desired reorganization and structuring of the scaffold material leading to tissues that are comparable to physiological ones. The UREPLACE bioreactor is a development that is used for the directed orientation of smooth muscle cells. The bioreactor system is made up of the following components (figure 2).

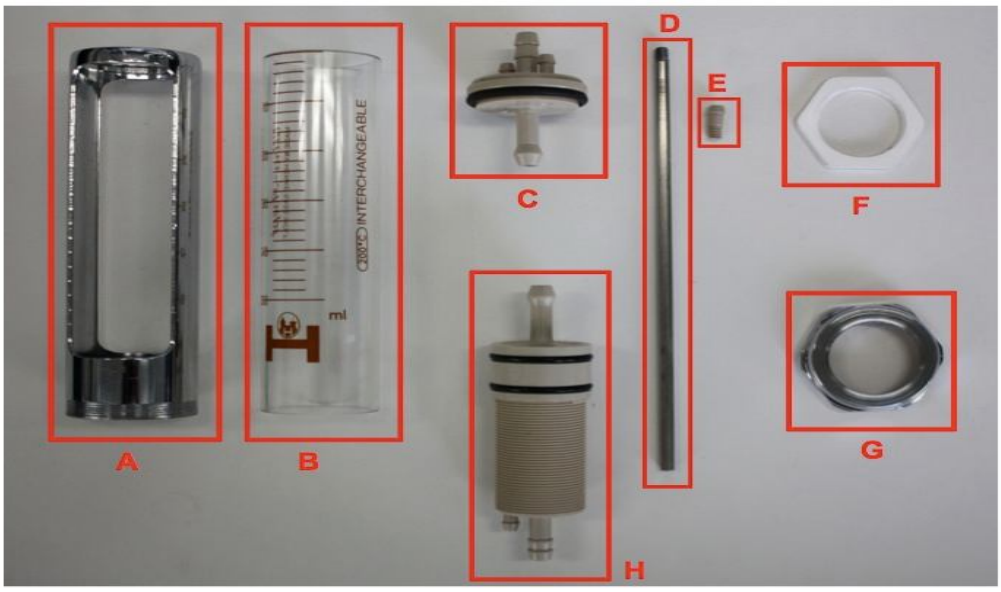

Figure 8: The UREPLACE bioreactor components; including: (A) metal housing, (B) glass cylinder, (C) front connector, (D) supporting metal rod, (E) small fitting, (F) adjusting nut, (G) stainless steel nut and (H) piston

It is the central element, where the OPTIMAIX sponge1 scaffold material is placed with its dimensions (l x d_i x d_o) 6 x 0.3 x 0.7 cm. Therefore, smooth muscle cells are seeded on the outer surface of the OPTIMAIX sponge1 scaffold material. A specific stimulation is performed leading to cell orientation.

Incubating system: The incubation system was designed for the application of the Ureplace bioreactor . It is based on a PMMA housing, modified with a heating system, a CO_2 fumigation chamber, two peristaltic pumps for the medium support, several holder for the bioreactor and actuators. Next to the incubator, there are additional devices like a pH-electrode in a flow chamber. Additionally, there is a LabBox as the central device for the data acquisition as well as for the control and regulation of the electrical devices. It is directly connected to a computer, where the LabVision software provides a user interface. That interface was designed for the application of the bioreactor system and it is used to set the most important values like the rotation angle, speeds, temperature, or the CO_2 concentration. Moreover, there are diagrams integrated, which show the most important measurement values. In case of any abnormality, it is possible to bring the system under control, again.

The bioreactor is prepared with smooth muscle cells on the outer surface of the OPTIMAIX sponge1 scaffold. In the following, the bioreactor is placed in the incubator and the scaffold is stimulated. After that, the procedure is repeated. A constant PBS flow prevents a contamination of the inner scaffold. The procedure is followed by the injection of the inner cells and distribution of these cells by the rotating unit.

Bioreactor: The new bioreactor was designed for the usage of cylindrical scaffolds OPTIMAIX Sponge 1. The main base is a metal housing where a glass cylinder is inserted. At the endings, a front connector and a piston, made of the thermoplastic polyether ether ketone, were applied.

These two parts have three connectors outward and one for the application of a scaffold projected to the inner volume of the glass cylinder. Two of the outer connectors were responsible for the surrounding of a scaffold with cell culture medium, while the inner one enabled a discharge

of cell culture medium in the inside of the scaffold. After inserting the front connector with an o-ring seal into the metal housing, the two o-ring seals of the PEEK piston were covered with vacuum fat. In a next step, it was dragged backwards into the glass cylinder with an aluminum rod, so that a scaffold can be fixed at both inner connectors. Then the glass cylinder is pushed into the metal housing and pressed onto the front connector with a stainless steel nut in order to tighten the bioreactor. Additionally, the piston has a thread, so that it can be adjusted correctly to the scaffold´s length.

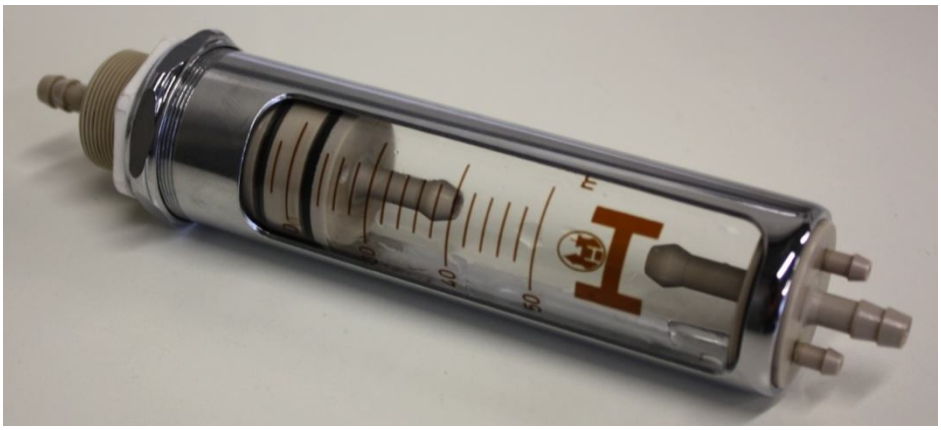

Figure: 9

All parts are autoclavable. Although there is liquid around the scaffold, a macroscopic observation of the bioreactor´s inside is still possible. To guarantee the necessary environment (temperature etc.) for cells, a customized incubator system was developed. For the evaluation of the bioreactor tightness, it was filled with distilled water and exposed to a 37 °C environment for 48 h in order to check the tightness.

Results: The tightness of the bioreactor was tested successfully after an incubation time of two days at 37°C. With the acquired temperature step response, the software based PI - controller was adjusted leading to a constant value of 37 °C ± 0.2 °C. A disturbance in terms of a complete Incubator opening was introduced into the system for 5 minutes, resulting in a temperature decrease to approximately 34 °C. After closing it again, there occurred a temperature overshoot up to a maximum of 39 °C, which decreases to 37 °C exponentially in 10 minutes. Moreover, the CO_2 concentration was adjusted to 5% with an software P - controller, based on acquired step responses. The pH - electrode step response showed a perfect function while measuring the pH - value of HCL, NaOH and PBS with a constant flow of 21 ml / min at 22.8 °C ambient temperature. Software stability was tested for 2 h successfully. The dynamical, cyclic stress within these two hours showed no fatigue of the system. The maximal measured pressure was about 1200 mbar. The software for the rotation works. The angle was set to 270°, but can be varied. Additionally, the program for the peristaltism operates correctly.

Conclusion: Although the system runs stable and the bioreactor is tight, first tests with cells have not been done yet. It has to be evaluated, if the smooth muscle cells will be oriented in the correct way. For the evaluation of the cell orientation, histological cuts have to be done, electron microscopic pictures have to be made and the gen expression of cytoskeleton proteins (in comparison to untrained cells) has to be checked. Moreover, the homogeneity of the cell distribution on the inner scaffold surface has to be evaluated, so that rotation angle and speed can be adjusted correctly. All in all, the bioreactor coupled with the new incubator system shows good tendencies to be successful.

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