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Review

Biological cardio-micro-pumps for microbioreactors and analytical micro-systems

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ABSTRACT

Bio-hybrid microsystems actuated by living cells, as micro-bio-actuators and micro-bio-pumps have been developed recently. In these devices biological cells may be powered without external energy sources and the movement or the contraction of muscle cells trigger off the flow of fluid (i.e. culture medium or blood) through microchannels in micro-multi-bioreactor systems. Isolated and *in vitro* cultured cardiomyocytes (cardiac cells) are the most promising bio-material, which can be used to design a micro-bio-pump/actuator. These spontaneously contracting cells are autonomously powered with glucose as an energy source without any external power supply or stimulus, unlike conventional micro-actuators/micro-pumps. Cardio-micro-bio-pumps/actuators are using collective, synchronous contracting forces of cardiac cells or cardiac cell sheets to drive the flow of fluid. The feasibility of building such actuators was demonstrated in a few examples of bio-hybrid microsystems actuated by single or sheeted cardiomyocytes.

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Contents

1. Introduction

It has been widely recognized that established substance safety testing of new developed compounds in the chemical, pharmaceutical and cosmetics sectors is poorly predictive to human long-term exposure. Recalls of substances from the market and a significant attrition rate at the level of bioactive compound development are the consequences. Currently available *in vitro* culture protocols for toxicology testing do not provide a fully acceptable *in vitro* model of human organ environment for repeated dose testing over long culture periods. Only several concepts nowadays combine a few different organotypic or tissue cultures within one micro-bio-environment system, thus allowing evaluating effects of chemical molecules, chemical active molecules, pharmaceutical drugs and

Abbreviations: ECM, extracellular matrix; PIPAAm, poly(N-isopropylacrylamide); PFC, perfluorochemicals (perfluorocarbons).

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Nomenclature

a	width of micropillar
C	curvature of substrate
E	Young's modulus
F	force
f	beat/oscillation frequency
H	height of micropillar
h	film thickness
I	moment of inertia
L	length of microcantilever array
Q	flow rate
R	radius of curvature
T	temperature
t	substrate thickness
ΔV	volume change in the chamber of micro-bio-pump
ν	Poisson's ratio of the substrate
Δx	displacement of top of micropillar
Δz	deflection of microcantilever array
σ	film stress

cosmetic compounds on tissues systems [1,2]. Consequently, engineers and scientists were faced with two main challenges at the beginning of this century [1]:

- to miniaturize organ/tissue culture space from millilitre to microlitre scale;
- to provide substantial sources of standardized human tissues.

It was obvious that further miniaturization of organ/tissue culture scale needs innovative realization ideas and radically new fabrication technologies.

It has been well known that every organ consists of multiple, functionally self-reliant, identical, micro- or millimeter scale structural units (from several cell layers up to a few millimeters), so-called sub-organoids [1–4]. Such organ specific units are the smallest building blocks of each human organ, including several cell layers up to 1 mm, which corresponds to a volume of microliters size. Each very small but sophisticated sub-organoid provides the essential functionality of one of the most prominent organ. Multiplication of micro-sub-organoid structures within a specified organ is a kind of "nature's risk management tool" to prevent total loss of functionality after partial organ damage [1]. Adult stem cell niches were recently found in many relevant tissues and organs of the human body. *In vivo*, these niches carry the capacity to fully regenerate nearby micro-organoids in case of loss or destruction [4,5]. *In vitro*, adult stem cell niches are the basis for the generation of such sub-organoids and form the basis of building artificial multi-micro-organoid systems [2,6].

The technology which needs to be integrated with culture technologies to overcome the gap in the miniaturization of dynamic bioreactors to micro-scale size is called micro-electro-mechanical systems (MEMS) technology, and is a business that crosses over multiple technologies to provide high performance micro/nano-systems in various applications, combining micro/nano-system research with micro-fluidic technology [1,6]. MEMS are at an early stage of their development cycle, but are already showing their great application possibilities. Nowadays, a lot of cell-based micro-system research takes place under this "lab-on-a-chip", "organ-on-a-chip" or a "micro-total-analysis-system" (μTAS) framework that seeks to create micro-systems incorporating several steps of an assay into a single system [6,7]. Integration of various process devices, cell culture systems and complex operations onto a multi-organ-on-a-chip is currently generating major interest due to the desirable characteristics of such system. "Living" micro-devices exhibit several distinct performance advantages including short diffusion distances, laminar flow regime, high interface-to-volume ratios and low heat capacities [7–9]. In other words, microchip devices provide several advantages for systems of cellular response analysis because the microfluidics inside the microdevice is appropriate to accommodate cells. New concepts in integrated miniaturized cell cultures systems also cope with the original human counterparts and satisfy the requirements of efficient long-term toxicology testing. Recently, bio-hybrid microsystems actuated by living cells as micro-bio-actuators and micro-bio-pumps have been developed in which biological cells may be powered without external energy sources and the movement or the contraction of muscle cells trigger off the flow of fluid (culture medium or blood) through microchannels in micro-multi-organ-on-a-chip systems [7,10,11].

Cardiomyocytes (cardiac cells) are the most promising biomaterial, which can be used to design a micro-bio-pump. These spontaneously contracting cells are autonomously actuated just with glucose as the only energy source without any applied electrical power supply or stimulus, unlike conventional microactuators/micropumps. Cardiomyocyte sheets were first produced in the field of tissue engineering to reconstruct or repair a heart without any artificial scaffolds. Sheets of cardiac cells can be harvested intact, and transferred or subcultured to various devices while maintaining their regular and robust contracting phenotype. In cardiomyocyte sheets macroscopic pulsation is visible, therefore they can also be used as an element of micro-bio-pumps [12,13]. Cardio-micro-bio-pumps (cardio-μ-bio-pump) use collective, synchronous contracting forces of cardiac cell sheets to drive the flow of the fluid. There are examples of hybrid (biotic/abiotic) devices, which consist of synthetic (polymers) and natural living (cardiomyocytes) materials [7,11]. The feasibility of such microstructures to force the flow of fluids in microchannels will be demonstrated below in a few examples of bio-hybrid microsystems actuated by single or sheeted cardiomyocytes.

2. Cardiomyocyte sheets

Cardiomyocyte based sheets were first produced for applications in tissue engineering and regenerative medicine [14]. The basic idea of cardiac cell sheets was to propose an alternative therapy to cardiac transplantation and to create a kind of smart bio-material to reconstruct or repair a human heart without any artificial scaffolds [15]. The contraction and viability of grafted myoblasts were confirmed in the early 2000s. Multipotent bone marrow cells or embryonic stem cells have been comprehensively investigated as possible candidates for human implantable myocardial cell source [15,16]. In early attempts, the therapeutic use of isolated cardiac cells was based on the well known concept of tissue engineering that 3-D biodegradable scaffolds are useful as alternatives for extracellular matrix (ECM) and that seeded cardiomyocytes will reform their native structure according to scaffold biodegradation. In myocardial tissue engineering poly(glycolic acid), gelatin and alginate have been used as biodegradable scaffolds [14]. In native myocardial tissue, cells are dense in comparison with other successfully scaffold engineered cell-sparse tissues (i.e. cartilage, vascular, heart valve). Cardiomyocytes in native heart tissue are also tightly 3-D interconnected with gap junctions mediated by the reciprocal exchange of biochemical mediators resulting in electrically synchronous beating which is attenuated in biodegradable scaffolds. It is also well known that confluent cultured cardiomyocytes beat simultaneously. When cardiac myocytes are fixed to a rigid polystyrene surface under conventional culture conditions their contracting motion is highly limited.

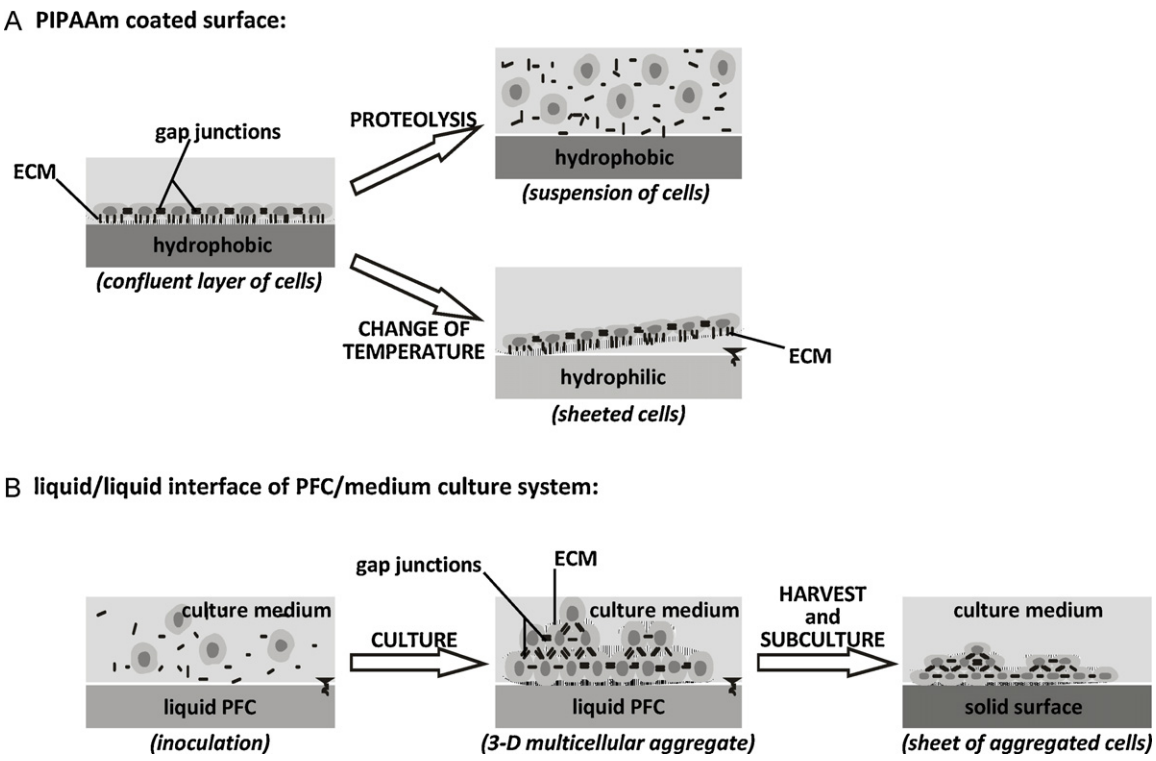

Fig. 1. Various types of cardiomyocytes cultivation: comparison of cell harvest due to detachment of the cells from surface with disruption of cell-to-cell junctions and ECM after protease digestion and release of cells growing on PIPAAm temperature-responsive polymer (based on [15]) (A); and cultivation of 3-D multicellular aggregates on PFC (hydrophobic)/culture medium (aqueous) flexible interface of cell culture system based on two non-mixing liquid phases (B).

Overlaying the sheets on an elastic, flexible material would increase the amplitude of simultaneous pulsating and minimize the negative interaction between confluent cell sheets and the surface of the culture vessel (dish or deep-well plate) [15]. The published cellular biomechanics experiments show that one rat cardiomyocyte can generate at least 1 μN of force (that is, force per cross-sectional area: 2.75 kPa) [17–20]. In this set-up's single rat cardiac cells were placed between a pair of carbon graphite fibers and the contracting force of single cardiac cells was estimated based on microscopically measured differences in location of graphite fibers. Higher forces (at the level of ~10 μN) were observed by Nishimura et al. [18] and by Yin et al. [19]. Yasuda et al. [17] reported that the native contracting force of cardiac myocyte can be significantly improved (by approximately 45%) by adjusting the composition of the culture medium for example by addition of isoproterenol (10 μM). Stronger contractile forces could also be produced by using contiguous multi-cellular cardiomyocyte sheets.

Cardiac cells can grow in pseudo-2-D cell sheets on temperature-responsive culture surfaces without any biodegradable solid or gel-based alternatives for ECM. The temperature-responsive culture surface allows controlling cell adhesion to the surface to which grafted cells are attached.

Poly(N-isopropylacrylamide) (PIPAAm) is one of the biologically non-toxic temperature-responsive polymers, which can be covalently grafted by electron beam to standard polystyrene dishes or deep-well-plates used in tissue cultures. The surface of a PIPAAm layer is hydrophobic at 37 °C but its surface changes reversibly to hydrophilic characteristics by simply lowering the temperature below 32 °C. Mammalian cells could adhere to and proliferate on the PIPAAm surface under typical culture conditions but detach from the surface when the culture is slightly cooled due to rapid hydration and swelling of the grafted temperature-responsive polymer [7,15]. As a result only the connection between cell adhesive proteins (i.e. fibronectin) and the PIPAAm surface is broken during harvesting and cells remain connected together as the pseudo-2-D multicellular sheet (Fig. 1A). By applying cell cultivation on the PIPAAm surface sheets of various cell types (i.e. vascular endothelial cells, keratinocytes, kidney cells, hepatocytes, skeletal myoblasts, mammalian and human cardiac myocytes) have been obtained [15,21].

When enzymatic proteolytic digestion is used to release cells, adhesive proteins are disrupted and each cell is released separately. In the case of using temperature-responsive polymer surfaces all cell-to-cell junctions are preserved and cells are easily harvested as a continuous cell sheet. Obtained viable cell sheets are composed of cells and ECM only without any artificial scaffolds. Adhesive proteins maintained underneath the cell sheet could play a desirable role as an adhesive agent in transferring cell sheets onto other surfaces (materials or cell sheets). Two techniques of cell sheet manipulation have been described depending on the cell type and properties of surfaces of destination materials. One is to manipulate cell sheets directly with forceps or pipettes after its harvesting is completed which results in shrunk and thicker constructs due to reorganization of cytoskeleton. Alternatively, support membranes [e.g. poly(vinylidene difluoride) (PVDF) membrane] can be used for preserving the morphology of cell sheets. Before cell sheet release from the PIPAAm surface, the support membrane is placed over the confluent cells. Then, the cell sheet physically attached to the support membrane is harvested, and can be easily transferred to its place of destination. The support membrane is removed but cells attach to the new surface by the remaining adhesive proteins [15,21].

Maintenance of 3-D structure and functional integrity of contracting cardiomyocyte sheets during fabrication is one of a key issue in their application in micro-actuators/micro-bio-pumps. Mostly cells are cultured on surfaces grafted with a thermo-responsible polymer, which is slightly cell adhesive at 37 °C but changes to be non-cell adhesive after cooling below 32 °C. A new

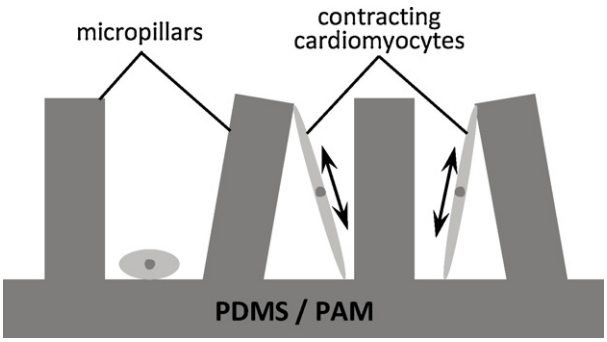

Fig. 2. The outline of the polymer-based micropillar actuators driven by the contractile force of cardiomyocytes attached to micropillars (based on [27,28]).

approach to cardiac cells sheets fabrication using a liquid/liquid interfacial area of perfluorochemical/aqueous systems for mammalian cells culture is possible [22,23]. Liquid perfluorochemicals (perfluorocarbons, PFCs) are characterized by a high solubility of oxygen, carbon dioxide and other non-polar gases, and have raised much interest in medical and technical applications [24–26]. One of the biotechnological applications of PFCs involves culturing 3-D animal cells aggregates on liquid/liquid interfacial area created between PFC and culture medium layers [22,23]. Animal and human cells adhere gently to the flexible PFC/medium interfacial area, spread and create multicellular aggregates or sheets (Fig. 1B). Such multicellular aggregates or sheets of animal cells can be easily collected without the use of aggressive proteolytic digestion and the 3-D structure of them can be preserved during subcultivation. Thus, sheets of cardiac cells, produced in such a 2-phase PFC-containing system will be an ideal starting point for inoculation of a cardio-micro-bio-pump diaphragm or membrane.

3. Single or few cardiomyocytes based pulsatile microactuators

3.1. Flexible hydrogel micropillar micro-bio-actuators

The concept of bio-micro-actuators using cultured cardiomyocytes coupled to polymer-based microstructures is to convert chemical energy of culture medium into mechanical energy of micropillars' movement. Two kinds of polymers are used as a material of micropillars: acrylic acid/N,N′-dimethylacrylamide (polyacrylamide gel, PAM) based hydrogel [27] and poly(dimethylsiloxane) (PDMS) [28], both studied by the Kitamori and co-workers. Fig. 2 presents the outline of polymer-based micropillar actuators. PAM hydrogel micropillars were chosen as model structures because their mechanics and Young's modulus are similar to that of living tissue and they can be easily driven, even by generally weak forces generated by the single cells. PDMS has a higher Young's modulus than hydrogel but is more cell-adhesive and is commonly used as a fundamental material of bio-hybrid lab-on-a-chip micro-systems. Both used micropillars differ in their dimensions: PAM items were 200 μm high in contrast to just 10 μm high PDMS micropillars. There is a significant difference in the height of microstructures in comparison with the size of cells (~10 μm) cultured on them. Both kinds of surfaces were modified with well-known cell-adhesion promoters: PAM with RGDS cell adhesion peptides covalently bounded to carboxyl groups of acrylic acid, PDMS with adsorbed fibronectin. Practically this was performed by pouring a suspension of collagenase-digested primary neonatal rat cardiomyocytes into culture dish containing the treated PAM or PDMS pieces. Spontaneous actuation of polymer-based micropillars was observed when the cultures have reached confluency. In both cases, deflections of micropillars were regular and the beat frequency was about 0.1 Hz in the case of PAM and 1.4 Hz in the case of PDMS microstructures [27,28].

The value of the contractile force generated by the cultured cardiomyocytes was estimated by the displacement of the PAM and PDMS micropillars by using a model based on Hooke's law [27,28]. For small deflections, a micropillar behaves as an ideal, simple Hookean spring, i.e. the deflection is directly proportional to the force generated by cardiomyocytes attached to the micropillar. Due to that both kinds of micropillars can be treated as beams composed of linearly elastic material and because they were under pure bending, the effecting force could be estimated as:

$$F = \left(\frac{Eat^3}{4H^3}\right)\Delta x \quad (1)$$

where F is the bending force, E is the Young's modulus of micropillar material (PAM or PDMS), a is the width of the micropillar, t is the substrate thickness, H is the micropillar height and Δx is the displacement of the top of the micropillar. The estimated values of the contractile force generated by cardiomyocytes in PAM and PDMS micropillar systems were 0.08 μN [27] and 3.8 μN [28], respectively, if forces of the cells were applied to the top of the micropillar. The authors also pointed out that stronger forces were needed for the displacement of the micropillars in the case of cardiomyocytes attached to the sides of the structure then to the top of the micropillar. The significant difference in the estimated values of the contractile force (44 times larger in PDMS system then in PAM set-up) has been discussed in details by Tanaka et al. [7,28]. Weak cardiomyocyte adhesion to PAM and various peptides/proteins used as cell-adhesion promoters were mentioned as main causes of the difference. Overall, PDMS was confirmed as a more accommodating base material to culture cardiomyocytes in comparison with PAM.

Zhao and Zhang [29] reported that cardiomyocytes attached to PDMS-based micropillar generate a stronger contracting force compared to previously published results of Morishima et al. [27] and Tanaka et al. [28]. But in their set-up cardiomyocytes were perfused with isoproterenol, which increases the inotropic, and chronotropic characteristics of cardiac cells. In this study forces were estimated as relation between the deflection of the top of circle-based micropillar (8 μm in height, 2 μm in diameter) and the lateral force:

$$F = \left(\frac{3EI}{H^3}\right)\Delta x \quad (2)$$

where I is the moment of inertia (varies proportionally to diameter of the cylindrical-shaped micropillar to the fourth power). The values for the contractile forces varied from 46.9 to 67.4 nN and the beating frequency from 1.5 to 3.68 Hz. The results obtained by Zhao and Zhang [29] and those discussed above as published by Morishima et al. [27] and Tanaka et al. [28], are difficult to compare due to lack of information about the origin and morphology of the cardiac myocytes used by Zhao and Zhang.

3.2. Bio-hybrid microcantilever microactuators

A cardiomyocyte based micro-system microfabricated PDMS-based microcantilever actuated with neonatal rat cardiomyocytes was presented by Park et al. [30]. Fig. 3 presents the outline of this biohybid microcantilever microactuator. The plain-surfaced arrays of the PDMS-based microcantilever (20 μm thickness) had five different sizes, from 50 to 300 μm wide. The length of each array was 5 times larger than its width. The microcantilevers were inoculated with rat cardiomyocyte suspension and cells attached firmly and spread on the surface of the microcantilever arrays. The first contractions of individual cells were observed after 24 h of culture. Synchronous contractions were observed after 48 h and they

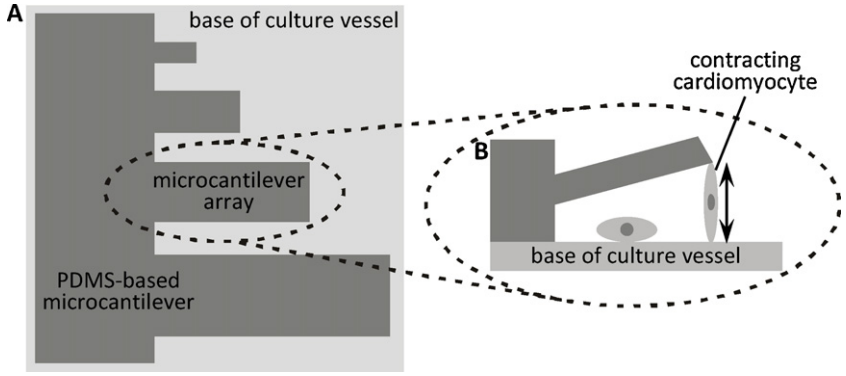

Fig. 3. The bio-hybrid microcantilever microactuator (based on [30]). The outline view of PDMS-based microcantilever (A) and cross-sectional view of microcantilever array actuated by attached contracting cardiomyocyte (B).

reached a maximum at 3–4 days of culture. The bending motion of the PDMS microcantilever can be exploited for micro-actuation. This very simple bio-hybrid system was used to study and measure the contractile forces generated by myocytes of the cardiac muscle. Both the lateral and vertical displacements of each micropillar were measured. To determine mechanical stresses generated by cardiomyocytes, a mathematical model based on the Stoney's equation was used:

$$C = \frac{1}{R} = \frac{6(1-\nu)h}{Et^2}\sigma \quad (3)$$

$$\Delta z = \frac{3(1-\nu)hL^2}{Et^2}\sigma \quad (4)$$

where C is the curvature of the substrate, R is the radius of curvature, ν is the Poisson's ratio of the substrate, σ is film stress, h is film thickness, L is the length of the microcantilever array and Δz is the vertical deflection of the microcantilever array. Finite element modeling (FEM) analysis has been used to find numerical solutions. The estimated stress from cardiomyocytes was from 2 to 5 kPa and similar to the value reported by Balaban et al. [31] (5.5 ± 2 kPa). After further improvement of the fabrication process of the 3-D polymer structure the stress generated in grooved microcantilever system reached up to 7–10 kPa [32,33].

3.3. Hybrid cell-motored micro-actuator

A cardiomyocyte-based asteroidal microdevice called "hybrid cell motor" was developed by Kim et al. [34]. Their concept was to apply patterned cardiomyocytes with 3-D morphology to force motion of the microrotor attached on a single or several cardiac cells (Fig. 4). In these experiments primary rat cardiomyocytes were placed in polymer (PDMS/polyethylene glycol based) micromolds/microwells with non-adhesive walls. The bottom of the micro-molds was coated with collagen to enhance cell attachment and survival in the microstructure. One to six cardiomyocytes were placed within a 75 μm circle of micro-molds. Interestingly two different types of cell morphology were observed during experiments: (i) pseudo-two-dimensional shape well known from animal cell cultures on solid surfaces and (ii) spatial 3-D structure observed when cells are culturing in ECM substitutes or scaffolds [34]. Finally, an asteroidal 3-D PDMS-based microstructure was poured on cardiomyocytes grown in the micro-molds. The microstructure had a 40 μm diameter hole in its centre, which fixes the microrotor to the cardiac myocytes.

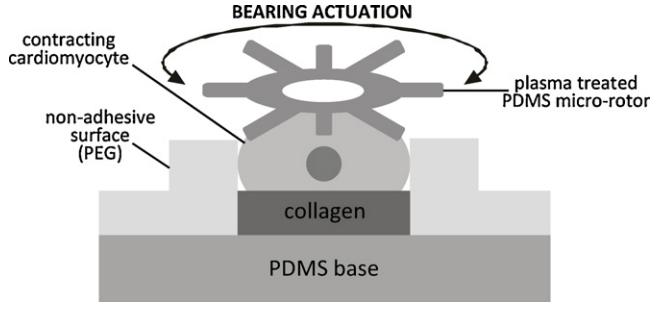

Fig. 4. The outline view of the hybrid cell motor powered by cardiomyocyte with 3-D morphology (based on [34]).

It was found that the contractile forces of cardiac muscle cells depend on the cardiomyocyte morphology. The cells with 3-D morphology were more active than cells with pseudo-2-D morphology. The beating frequency was also proportional to the number of 3-D-cells in the multicellular aggregate. Differences were noted in both, the beating frequency (3-D cells were over 20% more active), and the value of the contractile force (3-D cells were about 2 times stronger). The type of cell morphology varied depending on the micro-mold deepness. It was observed that cells poured in micromolds deeper than ~500 nm mostly gave rise to 3-D growth of cardiac muscle cells, but the authors suggested that also other parameters, not tested during these experiments, could cause cells to form 3-D aggregates. Finite element simulation and ANSYS simulation software technology (ANSYS, Inc.; USA) was used for the optimal design of the proposed bio-hybrid microactuator. It was estimated that the cardiomyocytes, which occupied one micromold generated about 20 μN per beating peak. Thus the power of cardiomyocytes with 3-D morphology estimated as the contractile force per unit area was about 9.05 kPa and was similar to those obtained by other measuring techniques [30–32].

4. Micro-bio-pumps driven by cardiomyocyte sheets

4.1. Micro-bio-pump based on a pulsating cardiomyocyte sheet

Cardiomyocyte sheets establish electrical communications between neighbouring cells, which result in their strong and collective synchronous macroscopic pulsation. More practical and stronger actuating micro-bio-pumps/actuators could be created by exploiting long surviving and simultaneous beating sheets of cardiac myocytes [15]. A bio-actuated pump on a microchip powered by a cultured cardiomyocyte sheet and cell-coupled fluid mechanical motion was first demonstrated by Tanaka et al. [35]. A schematic design of their device is shown in Fig. 5.

The square-shaped (2×2 cm) microchip consisted of four PDMS-based elements: a push-bar (4 mm top and 2 mm bottom diameters), a chamber (3×0.5 mm) layer, a diaphragm membrane, and a microchannel (0.2×0.2 mm) layer with two holes (0.2 mm

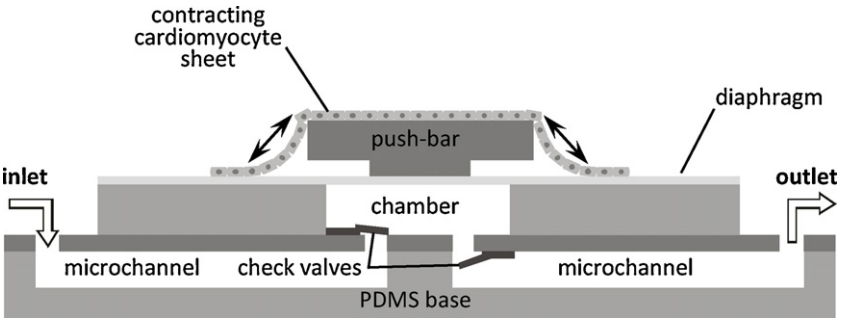

Fig. 5. The cross-sectional outline view of the bio-actuated pump on a microchip powered by cardiomyocyte sheet (based on [35]).

diameter) for the fluidic transfer. The push-bar was covered by the square-shaped (1 × 1 cm) cardiomyocyte sheet, which was also attached to the diaphragm. The complete device was immersed in cell culture medium to supply the cardiomyocyte sheet with glucose and oxygen. The temperature was maintained at 37 °C during all experiments. The flow of the medium through microchannels was driven by contracting forces of the sheet of cardiac cells attached to the fibronectin coated push-bar. The thin (10 μm) PDMS diaphragm and push-bar transmit the force created by cardiomyocyte sheet to the elements of fluid and produce an oscillating flow in the microchannels. The fluid flow in the microchannels was analysed in two systems: (i) in a bio-actuated pump without any kind of check valves and (ii) in the same bio-chip system but supported with two (inlet and outlet) polyimide micro-check valves (25 μm thickness) [35]. Fluid motion has been demonstrated with spherical (1 μm in diameter) polystyrene tracking particles dispersed homogenously in the cell culture medium and their movement was observed directly using a phase-contrast microscope. In the case of non-directional fluid pumping, the spontaneous oscillating fluid motion in microchannels was noted after cardiomyocyte sheet attachment on the push-bar. It was a result of repeated, regular pulsating stroke movements of the cardiac cells. The fluid oscillating frequency was 0.7 Hz with 150 μm maximum linear displacement of tracking particles and the oscillations were observed for about 7 days in the system with the culture medium changed every 24 h [35].

The expected theoretical flow rate (Q) by using ideal check valves to regulate the flow direction of the pump without reflux was estimated from the following equation:

$$Q = f\Delta V \tag{5}$$

where f is the oscillation frequency and ΔV is the volume change in the chamber. For estimated value of ΔV equals 5.6 nL the flow rate Q amounts 0.24 μL min^{-1}. However, the real microchannel flow rate estimated for directional fluid pumping which was realized in the bio-chip system supported with the cantilever-type micro-check valves was only approximately 2 nL min^{-1} and thus two orders of magnitude lower than estimated for a theoretical, non-reflux bio-chip supported with ideal micro-check valves [35]. The improvement of check valves and the use of integrated multilayered cardiomyocyte sheets were pointed as possible ways to enhance the bio-mechanical properties of hybrid micro-pumps actuated with sheets of cardiac myocytes.

The temperature changes could be used as a simple way to control the bio-pump/actuator performance. Influence of temperature on cell-based fluid pumping has been studied by Tanaka et al. [35]. First, the incubation temperature of the device was decreased from 37 to 30 °C within 1 h and subsequently the temperature was increased from 30 to 40 °C over about 0.5 h. The authors observed that the rise of incubation temperature increases the frequency of cardiomyocyte-based contractions, while the measured displacement of tracking particles decreased slightly [35]. In the case of the temperature decrease, the cells beat slower (f = ∼0.2 Hz at T = ∼33 °C and lower) but the motion of tracking particles was at least 20% higher. Thus it was concluded by the authors that external control of fluid pumping and performance of bio-pump/actuator is possible by simply modifying the temperature.

4.2. Hybrid micro-pump actuating by cardiomyocytes attached to dome-shaped diaphragm

A hybrid micro-pump actuated by the up-down motion of a dome-shaped cardiomyocyte-polymer diaphragm composite was described by Park et al. [36]. The concept of a dome-shaped membrane has been recognized as critical since a plain membrane did not produce a brisk up-down motion with the beating of cells on the surface. Thus, the key element of this device was a thin dome-shaped polymer composite consisting of a PDMS membrane (8 μm) with a Cr/Au (800/150 Å) layer and 50 μm central deflection. A self-organized confluent layer of cardiomyocytes was formed when cells were directly seeded on the diaphragm (Fig. 6). The dimensions of the actuating element (800 × 800 μm) and the overall size of the device (max. 15 × 30 mm) led to the conclusion that this device is a real micro-pump/actuator in contrast to the described above the bio-actuated micro-chip developed by Tanaka et al. [35].

The top layer of this micro-device was a PDMS-based microchannel layer supported with nozzle and diffuser as passive micro-valves. The direction of the flow was controlled by the fluid resistance in the combination of nozzle and diffuser elements. The opening angle of the nozzle/diffuser was 10°, their inlet/outlet necks were 100/500 μm wide and both micro-valves were 2.3 mm long. The dome-shaped polymer-metal diaphragm was situated at the centre of the bottom side of chamber. The outside (Cr/Au side) of the diaphragm was coated with fibronectin and gelatin and then neonatal rat cardiomyocytes were seeded on it to create the confluent monolayer of cardiac cells. When the hybrid diaphragm was actuated by the contractile pulsation of spontaneously and autonomous beating cardiac myocytes, the volume of microchannel chamber shrank and recovered periodically, resulting in a fluid flow. Regular beating of the cells was started after 2 days from inoculation and it was continued for the next 5–6 days of the experiment with culture medium exchanged in 48 h intervals [36].

The fluid motion in the microchannel was monitored by tracking 2 μm spherical polystyrene beads with a microscope and digital camcorder. The actuating frequency of the diaphragm was between 0.2 and 0.4 Hz and its vertical movement was about 8 μm. A finite element model [30] and numerical methods were used to estimate the net flow rate, which was formed in the direction intended by the nozzle/diffuser configuration of the device. The calculated theoretical mean flow rate was about 7 nL min^{-1} and thus one order of magnitude larger than the rate of 0.23 nL min^{-1} estimated by tracking the motion of beads [36]. There are several reasons of dif-

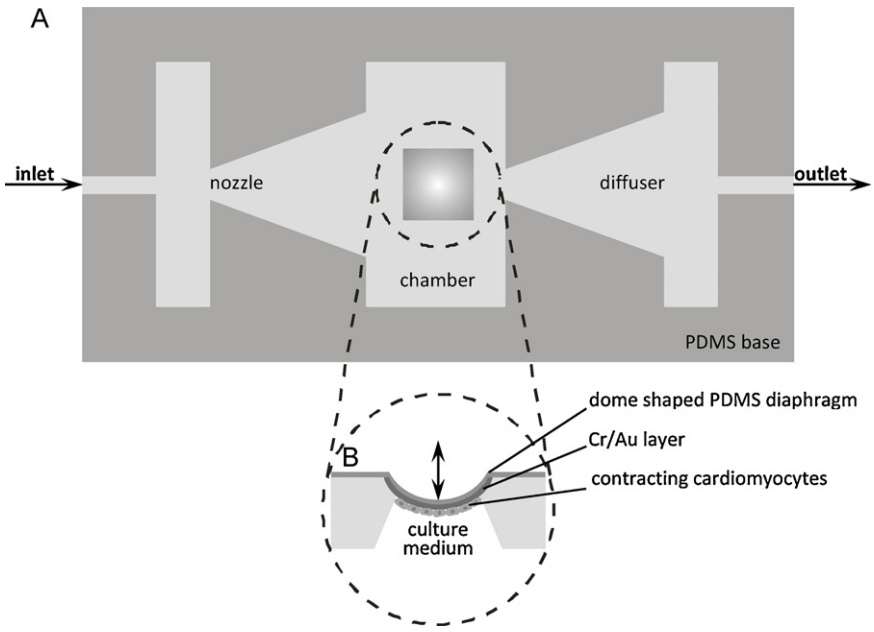

Fig. 6. The micro-bio-pump actuated by the dome-shaped hybrid diaphragm (based on [36]). The outline view of micro-device (A) and structure of dome-shaped hybrid diaphragm (B).

ferences between the theoretical and real values of the flow rate. The irregularly deformed shape of diaphragm during the contraction moment is one of them. Another one is the inequality of forces generated by cells attached to the diaphragm.

4.3. Micro-spherical heart-like cardio-micro-bio-pump powered by a cardiomyocytes sheet attached onto an elastomer hollow sphere

A micro-spherical heart-like pump powered by spontaneously contracting cardiomyocyte sheets was presented by Tanaka et al. [37] (Fig. 7). The device was fabricated by wrapping a beating sheet of cardiac myocytes around a PDMS-fabricated hollow elastomeric micro-sphere fixed with inlet and outlet ports. A micro-bio-pump was immersed in the cell culture medium and fluid oscillations in a capillary connected the hollow sphere were induced by the synchronously pulsating cardiac cells. The authors pointed out that the design (the hollow sphere) is an optimum structure and represents fundamental function of a heart-like item, similar to the earliest primitive heart (one chamber, no atrium or valves) as it is known in physiology of annelids [37].

A PDMS-based hollow sphere was utilized as a base and a cardiomyocyte sheet was rolled onto it after improvement of cell-adhesive properties of the surface realized by fibronectin adsorption. A confluent circle shaped sheet (1.5 cm in diameter) of neonatal rat cardiac myocells was used as a generator of contractile forces. The inside/outside diameter of the final heart-like micro-pump was 4.8/5.3 mm with about 250 μm thin elastomeric sphere. The device was supported with a Teflon®-based capillary (200/400 μm of inside/outside diameter) fixed with a hollow sphere by using epoxy glue [37].

Oscillating fluid motion in the capillary was monitored by polystyrene tracking particles. The fluid oscillating frequency measured at 37 °C was 0.4 Hz and the maximum observed linear displacement of tracking particles was 70 μm. The device worked for 5 days continually with culture medium exchanged in 24 h intervals [37]. The expected flow rate (Q) was estimated by the same methods (Eq. (5)) as described earlier by Tanaka et al. [35]. The beat frequency (f) was measured as 0.6 Hz at 37 °C, the volume change in the sphere-chamber per displacement (ΔV) was 2.2 nL and value of the expected flow rate ($Q = f\Delta V$) was 0.047 μl min^{-1}. The expected flow rate was smaller than the calculated theoretical flow rate for the cardio-micro-bio-pumps described above [35,37]. This is probably due to the lack of any valves, which could direct the flow of the fluid. The thickness of the PDMS-based hollow-sphere membrane, being 25-times larger than membranes used in other devices, maybe the reason for the low flow rate.

5. Other cardiomyocyte-based non-fluidic micro- and millimeter-scale devices

Single or sheeted myocytes of cardiac muscle were also used for assembling bio-powered hybrid micromechanical devices, i.e. a coiled strip oscillator, a helical linear actuator, or motile robotic walking/swimming actuators [38,39]. Some of them are schematically presented in Fig. 8. These milli- and centimeter-scale constructs were not considered as real micro-pumps but they also could be classified as bio-hybrid cardio-micro-actuators despite the fact that they perform functions as diverse as gripping, walking or swimming. These micro-bio-actuators are also versatile and may lead to integration of cardiomyocytes with a variety of diverse micro-structures.

A hybrid self-actuated walking micro-robot powered by self-assembled Sprague-Dawley rat cardiomyocytes was described by Xi et al. [38]. In this trial the cardiac cells were attached directly to the thin (20 mm/300 nm) film of Cr/Au-based Σ-shaped "leg" (138 μm long, 40 μm wide) without any hinges (Fig. 8A). The nanometer-metal film resulted in bending movement of the mobile "leg". The bent metal film stores energy when the muscle contracts, which contributes to the relaxation of the "leg". As front part of the "leg" bent with contractile force of attached cardiomyocytes, the walking micro-robot moved forward. The back part of the "leg" was designed to contract just partially so its relaxation balanced the micro-construct and resulted in forward movement of the walking micro-device. The contraction of the self-assembled cardiac cells powers the motion of the hybrid micro-robot. The contraction force must exceed all opposing forces such as drag force and friction which dominant in an aqueous medium. The power of the used cardiomyocytes attached to the Σ-shaped "leg" of the walk-

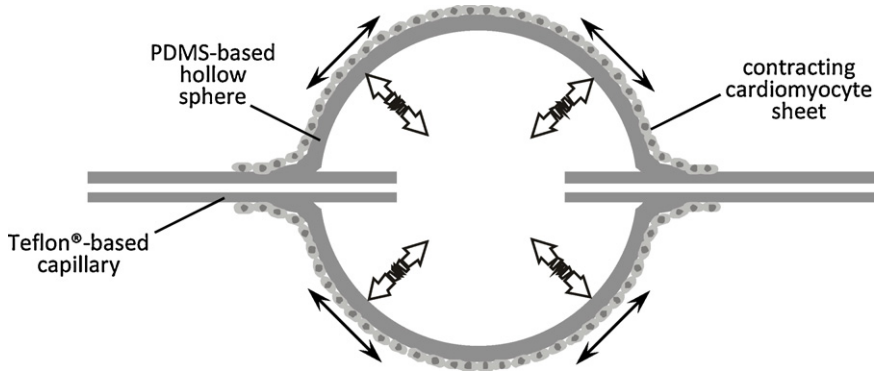

Fig. 7. The cross-sectional outline view of the micro-spherical heart-like pump (based on [37]).

ing micro-robot estimated as the contractile force per unit area was about 14 kPa, the average "step" frequency was 1.8 Hz and its average size was 25 μm. The bio-hybrid micro-construct could walk with a maximum speed of 38 $\mu m\,s^{-1}$ [38].

Several centimeter-scale bio-hybrid proof-of-concept 3-D actuators/oscillators and soft robotic motile devices driven by neonatal rat ventricular cardiomyocytes were presented by Feinberg et al. [39]. The very thin (14–60 μm) PDMS film was used as an abiotic base of micro-constructs and the film thickness was controlled by varying the viscosity of the PDMS pre-polymer and the spin-coating speed. The desired shapes of PDMS scraps were manually prepared with a scalpel and they spontaneously adopted a 3-D conformation determined by the film properties or were fashioned to create more complex 3-D shapes. The PDMS film allows sculpting of functional 3-D forms and restoring elasticity of designed shapes of constructs. The pseudo-2-D sheeted cardiomyocytes provide spontaneous (or electrically induced) contractile function. Such bio-hybrid 2-layered PDMS-cardiomyocyte sheet was named by the authors to be muscular thin films [39]. Contraction force of the attached cardiac cells caused a decrease of the radius of film curvature and modified shape of the PDMS scraps. Long rectangular strips with cardiomyocytes aligned along their length oscillate between a loosely coiled form (diastole of cardiac cells) and tightly rolled state (systole) during cyclic movements (Fig. 8B). Finally, the volume of the formed cavity was reduced during the contraction process. The systole phase was faster than relaxation and the stress of this coiled structure was estimated to be at least 15 kPa [39]. The difference in length of contraction and relaxation phases could be utilized in valveless pumping of viscous fluids similar to the embryonic vertebrate heart tube by peristaltic action. A helical linear hybrid micro-actuator capable of cyclic and axial extension and rotation was obtained by aligning cardiomyocyte fibers on a very thin PDMS-based strip (Fig. 8C). This structure spontaneously adopted a helical conformation (compared by the authors with a "paper towel tube"), where the pitch was a function of the angle between the longitudinal axis of the cardiomyocyte fibers and the midline of the PDMS ribbon. The axial extension for about 300 μm and the circumferential rotation of about 50° were estimated as characteristic parameters of the helical hybrid micro-actuator and such construct was proposed as a simple suction mode micro-pump. Feinberg et al. [39] additionally presented a soft robotic cardiomyocyte-based gripper. In this device the concave surface of an arched-bent rectangular thin strip of PDMS film was covered with a lengthwise-aligned cardiomyocyte sheet (Fig. 8D). The ends of the gripper come together until they touch and stop due to the contracting force of the cardiac cells. Finally, the cardiomyo-gripper was switched from an open state to a closed state during contraction and the stress generated by such type of cardio-actuator was about 25 kPa. Electrical stimulation (10 V amplitude, 10 ms pulse-width, 0.25–5.0 Hz frequency) was used to control the systolic bending of the gripper and to hold the opposite ends of the PDMS film at a prescribed distance [39]. Finally, also two kinds of bio-hybrid cardio-robotic muscular thin film motile constructs: a walking myopod and a swimmer were also developed by Feinberg et al. [39]. Fig. 8E presents the idea of a locomoting myopod device formed from a triangular thin PDMS film by folding the tip of the triangle into a loop reattached midway along the height to create a kind of "footpad". The bio-hybrid myopod walked spontaneously along the bottom of a Petri dish in a directed path but a constant velocity (about 133 $\mu m\,s^{-1}$) was only obtained when the contraction rate

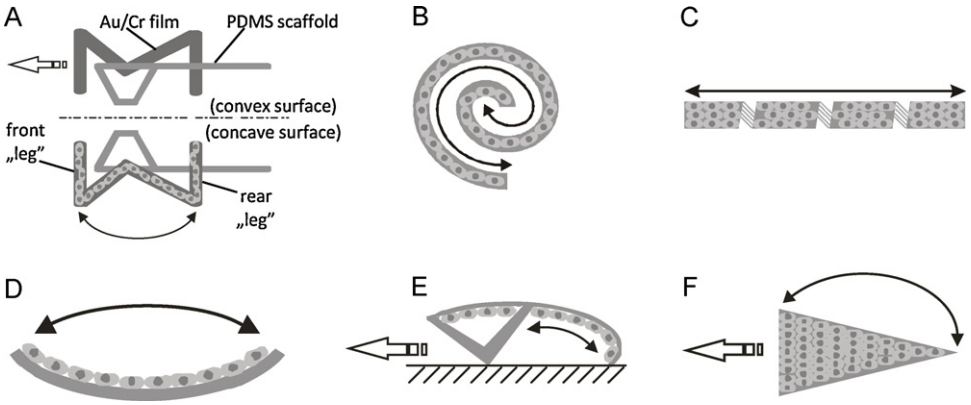

Fig. 8. Cardiomyocyte-powered bio-hybrid micromechanical non-fluidic devices (based on [38,39]). The self-actuated walking cardiomyocyte-based micro-robot [38] (A). The soft cardiomyocyte-based robotic actuators with diverse functionality [39]: the coiled-spring actuator (B), the helical linear actuator (C), the arched-bent gripper (D), the walking myopod (E) and the isosceles triangular swimmer (F). The black arrows symbolize the contracting forces of cardiomyocytes, the white arrows show the move directions of constructs.

Table 1
Comparison of micro-pumps and micro-actuators actuated by single/few cardiomyocytes or cardiomyocyte sheets.

Micro-pump/aerator	Generator of contractions	Origin of cardiac cells	Device dimensions	Generated force or presure	Measured flow rate	Contraction frequency	Time of activity	Ref.
Hydrogel-based micropillar micro-bio-actuator	Single/few cardiomyocytes	Neonatal Wistar rat heart	$\phi = 4$ mm	0.080 μN	Not measured	0.1	Not mentioned	[27]
PDMS-based micropillar micro-bio-actuators	Single/few cardiomyocytes	Neonatal Wistar rat heart	10×10 mm	3.8 μN	Not measured	1.4	Not mentioned	[28]
	Few cardiomyocytes*	Not mentioned	Not mentioned	0.047–0.067 μN	Not measured	1.5–3.7	Not mentioned	[29]
Bio-hybrid microcantilever micro-actuators	Single/few cardiomyocytes	Neonatal Sprague/Dawley rat heart	Not mentioned	2–5 kPa	Not measured	Not mentioned	4 days	[30]
	Single/few cardiomyocytes	Neonatal Sprague/Dawley rat heart	Not mentioned	4–7 kPa	Not measured	Not mentioned	4 days	[32]
Hybrid cell-motored micro-actuator	Single/few cardiomyocytes	Neonatal Sprague/Dawley rat heart	$\phi = 0.075$ mm	9.05 kPa	Not measured	0.8	4 days	[34]
Micro-bio-pump based on a pulsating cardiomyocyte sheet	Cardiomyocyte sheet	Neonatal Wistar rat heart	20×20 mm	Not measured	2 nL min^{-1}	0.7	7 days	[35]
Hybrid micro-pump actuated by cardiomyocyte sheet attached to dome-shaped diaphragm	Cardiomyocyte sheet	Neonatal Sprague/Dawley rat heart	15×30 mm	Not measured	0.226 nL min^{-1}	0.4	6 days	[36]
Micro-spherical heart-like cardio-micro-bio-pump	Cardiomyocyte sheet	Neonatal rat heart	$\phi = 5.3$ mm	Not measured	47 nL min^{-1}	0.6	6 days	[37]

* Isoproterenol-based stimulation of cardiomyocytes.

of cardiomyocytes was remotely controlled by electrical field stimulation (10 V amplitude, 1 Hz frequency) [39]. The relative velocity of Feinberg's monopod (about 2.7 lengths min^{-1}) was similar to that reported by Xi et al. [38] for their cardiomyocyte-powered micro-construct (about 3.7 lengths min^{-1}). Fig. 8F schematically presents the triangular swimmer designed by Feinberg's group [39]. The swimming millimeter-scale device was realized by aligning a cardiomyocyte sheet parallel to the height of the 30-μm-thick PDMS isosceles triangle. The microarchitecture of the cardiomyocyte sheet was found to be critical for potentiating the mobility of the swimming robot. The swimmer powered by spontaneously contracting sheeted cardiomyocytes with anisotropic morphology (average velocity of 30 μm s^{-1}) moved about 5 times faster than the device powered by the isotropic myocardium (average velocity of 10 μm s^{-1}). A maximum velocity (400 μm s^{-1}) was achieved in the case of remote electrical stimulation (10 V, 1 Hz) of the anisotropic swimmer [39].

6. Conclusions

So far only few prototypes, which can be named as cardio-micro-bio-pumps/actuators have been described in literature. Basic properties of those devices have been compared in Table 1. They are interesting actuators working only with chemical energy input and mechanical force generation output. Cardiac myocytes, the most heavily working muscle cells in the living bodies, were used as generator of the force, which triggers the flow of fluid in microchannels. Such trials are of great interest for applications encompassing microfluidics, micro-multi-bioreactor systems and high-throughput assays. But none of the presently published cardiomyocyte based devices were checked for long-time contraction or were exploited for any practical applications to our knowledge. Also no biological based actuator was applied so far as a functional part of an organ-on-a-chip device. One of the basic aims of alternative (non-animal) testing methods mentioned in the European Commission report on alternative testing strategies and in the well-known "three Rs" concept is to create a functional and fully acceptable human organ model for repeated dose testing over long culture periods [40]. Efficient substance safety/toxicology testing is done for 14, 28, 90 days and 1 year. Human cardiomyocytes seem to be suitable to create such bio-hybrid device working for a 1 year period utilizing the ability of cardiac cells to work for a life.

Published results show that single or sheeted cardiomyocytes have the capability to generate forces in the order few nN. These values are far larger than those obtained in other micro- or nano-actuators such as molecular motors or laser tweezers which typically generate only pN-order forces [41–43]. Isolated aggregates of cardiomyocytes show different contractile activities depending on their morphology [34]. This suggests that a tight control of cell growth and shape would be necessary for improving the mechanical and dynamical properties of cardiomyocyte-based micro-pumps/actuators. In certain applications, cardiomyocyte aggregates could be suitable as microactuators to drive objects (i.e. molecules or particles) or fluids in a microspace. The flow rates presently generated in cardio-micro-bio-pumps may be sufficient for special bio-medical applications such as local drug delivery systems or stimulation of damaged nerves [7,44]. More efficient micro-bio-pumps might be developed which overcome the limitations in single/few-cells mechanically coupled cultures by exploiting the potential of much more robust multicellular cardiomyocytes sheets. Such self-actuated devices should be useful to intensify mass transfer in micro-chip based systems with-

out any electrical input. Thus one may speculate that future generations of cardio-micro-bio-pumps could be applied as a functional component of multi-organ-on-a-chip devices and bio-hybrid microsystems. The challenge will be to provide the cells with a nutritional diet, which keeps them alive and working for longer periods. More sophisticated methods to produce substrates with more uniform cell-attachment and mechanical properties are also required for all proposed applications.

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