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#### **Biohybrid Motile Bots Neuromuscular Actuation**

Аннотация. Основная цель работы – анализ метода приведения биороботов в движение путем создания функциональных нервно-мышечных соединений. В статье рассмотрены предыдущие проекты, использующие мышечные ткани для движения роботов и основные этапы создания биогибридного робота типа «пловец», приводимого в движение посредством нервно-мышечных структур. По результатам анализа сделаны выводы касательно используемых методов.

**Ключевые слова:** пловец, биогибридные системы, биологический силовой привод, нервно-мышечные соединения.

**Abstract.** The main purpose of the work is to analyze the method of driving biorobots by creating functional neuromuscular connections. The paper discusses previous projects using muscle tissues to move robots and the main stages of creating a biohybrid robot like a "swimmer" driven by neuromuscular structures. Based on the results of the analysis, conclusions were drawn on the methods used.

**Key words:** swimmer, biohybrid systems, bioactuator, neuromuscular junction.

#### **Introduction**

Biohybrid robots, which are a system of mechanism and living cells, are a popular research topic. Engineers seek to use the technical solutions developed by nature itself in billions of years of biological evolution.

Biohybrid machines, developed through the integration of artificial and biological components, are emerging as platforms to understand and synthesize the processes that drive structure, function, and behavior in biological systems. The biohybrid approach is appealing in engineering due to unique advantages that biological components can offer, such as high energy efficiency, stimuli-responsive adaptation, and the ability to self-organize. Moreover, biohybrid systems may allow us to decipher fundamental design principles of natural organisms in simpler and controlled in vitro settings. These principles can in turn be applied to develop artificial, bioinspired designs.

### Previous mobile robot projects

The first project, created in 2012, presented an artificially created jellyfish. It was driven by rat cardiomyocytes. A distinctive feature of tissue consisting of such cells is the ability to shrink even when grown separately from the body.

During the tests, the muscles shrunk under electric current and the elastic substrate material (polydimethylsiloxane) returned the dome to its previous position. Thanks to this change in dome volume, the jellyfish moved [2].

Later that year, another mobile biohybrid robot project was introduced. Experts from the University of Illinois presented a walking seven-millimeter robot. Under the influence of synchronous muscle contraction, the robot leg bends and, according to the principle of the lever, pushes the device forward [5].

In 2014, the same group of scientists introduced walking robots. Unlike the predecessor, the basis of this robot is skeletal muscle, which can be controlled. In this case, they are controlled by electrical pulses [1].

Previous work on floating biorobots copied sperm movements and consisted of rat cardiomyocytes. However, such a system could not be managed. It proved the possibility of creating similar floating machines, but it had no practical benefit [3].

A new work published in October this year introduced a new swimmer. This time it consisted of conventional skeletal muscle tissue, however, it had innervation to neurons sensitive to light [4].

### <u>Method of neuron-muscle coculture</u>

The main feature of this robot is the co-culturing of muscle and nerve cells. According to the authors of the draft, with joint growth, functional neuromuscular junction will be formed. To realize this idea, some moments were envisaged in the framework design [4].

First, the frame is made free standing so as not to limit movement of the neuromuscular junction. All previously presented projects had either a flat frame or a volume but fixed.

Second, since co-culturing is contemplated, two temporary seeding molds are used to create a cavity around the legs.

The myoblast-laden gel compacts during the first 2 d in culture, and this compaction is constrained by the scaffold legs, leading to the formation of a muscle strip bridging between the 2 legs.

Then neurospheres containing motor neurons are introduced into the cavity with muscle strip. They were obtained by directed differentiation of mouse optogenetic embryonic stem cells.

. During the first 3 d of coculture, we observe neurite outgrowth emanating from the neurosphere into the surrounding extracellular matrix. Neurite outgrowth was remarkably biased toward the muscle strip with very few neurites growing in other directions, most likely due to soluble factors secreted by muscle cells which have been shown to enhance neurite outgrowth in culture. A stimulation/inhibition assay was used to test the functionality of neuromuscular junction. D-tubocurarine (curare) was an inhibitor of neuromuscular junction [4].

It is worth noting that this is the first experience of co-culturing and the formation of functional neuromuscular units. All projects had previously used tissue monoculture and had not been faced with the problem.

### <u>Computationally Guided Design of the Swimmer</u>

The model of the swimmer presented by the authors is the first to use a volume and free-standing frame. So in modeling, this design had to solve several important problems.

In addition to the features dictated by the feature of neuromuscular junction development, it was worth taking care to stabilize the swimmer against roll and pitch, produce pure traction and develop strong swimming movements.

A flat head model was chosen as a measure to stabilize the swimmer, and a biomimetic harness model was used to provide pure traction as a prototype of the harness in the swimmer. After the completion of the model, drawings of two variants of the future swimmer were created. The first option carried one harness and was similar to the previous project of this team. The second version carried two bundles.

In the course of computer modulation, a better configuration was shown by the two-way variant. Also during this modulation, it was confirmed that the optimal velocity can be achieved at the intermediate tail length. At the end of the modulation, the stability of the selected model was tested under the influence of experimental uncertainties [4].

### Practical tests

Based on the models above, a dual-cuticular swimmer was created and placed in a liquid medium. The shooting was used to report movement in the environment.

In a state of rest, drift caused by disturbances in the environment was recorded. Under the influence of light, neuromuscular junction began to shrink, forcing the harnesses to oscillate and advance the swimmer. After some time, the speed of movement decreased, which was caused by the physical winding of the system. After a short break, the initial speeds were restored.

Speed was expected to be quite low. This is due to both the size of the robot itself and the viscous resistance of the volume head. The readings and characteristics of the framework are optimized for a given neuromuscular drive. Several strategies can be implemented to reduce drag and improve thrust, hence achieving higher speeds. Miniaturization and optimization of neuromuscular design to increase compactness and force output are crucial to these strategies [4].

### **Conclusion**

The presented model illustrates a new path in the development of biohybrid machines.

The general model of construction of free-standing scaffolds and methods of tissue cocultivation, derived in this work, is fundamentally new and effective.

A number of factors can be considered as unique warrantor of model operability.

Such factors include the synergy of the biological and artificial components, the coincidence of the results predicted in the modulation, regarding the speed, stability, and influence of the tail length and the angle of their opening, with those obtained directly in practical testing.

In the future, such robots will be smaller and will be able to speed up. One possible application is medicine.

Such robots will be able to play the role of antibodies or tiny surgeons. They can also be used as a diagnostic tool. It is even possible that biohybrid robots can be used to interfere with the genome.

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