A bio-inspired approach towards the development of soft amoeboid microrobots

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Abstract—Innovative soft robots can be developed at the microscale by taking inspiration from the biophysical descriptions of amoeboids. In this study, we fabricated active hydrogels as possible structural and functional materials in order to achieve locomotion at the microscale. The prepared hydrogels contain a moiety that exhibits color changes through the reversible redox of ruthenium complexes, driven by the Belousov-Zhabotinsky reaction. Such chemical oscillation in the hydrogel interact with the polymer matrix to cause mechanical ones, that can be exploited as means of actuation and possibly to obtain locomotion. Furthermore, in this study the self-oscillating hydrogels are compared with biological materials to validate their choice in the development of a self-propelled stimuli-responsive microrobot inspired by amoeboids.

I. INTRODUCTION

Amoeboids are, in biology, life-forms characterized by an irregular shape and by the ability to change their shape at will, as is reflected in the etymology of the word, which derives from the Greek amoibè (αμοιβή), meaning change. Most amoeboids belong to the Kingdom Protista, a sister group to animal and fungi and one of the earliest branches from the last common ancestors of all eukaryotes [1]. The most widely known amoebozoa are unicellular, but this group also includes slime moulds like Physarum polycephalum or Dicytostelium discoideum, that can have a multicellular life stage when its unicellular amoebas are starving. Members of the Kingdom Protista can greatly vary in size, from few tens of micrometers to tens of centimeters.

Amoeboids become attracting from an engineering point of view after the observation that their peculiar way of locomotion allows them to move in different unstructured environments, each imposing different constraints on the organism. An example is represented by the parasitic amoeba Entamoeba hystolitica that can move from the intestinal epithelium through the bloodstream to colonize the liver [2]. A detailed phenomenological description of amoeboid locomotion is beyond the scope of the present article, the interested reader can refer to key works on the topic [3-5]. A further point in favor of our interest in amoeboids is their general lack of specialized structures responsible for locomotion, which arises from interactions between different portions of the cytoplasm and the environment. These interactions have, in the literature, been alternatively studied from an “internal” point of view [6-8], focusing on the molecular dynamics of the cytoskeleton, and from an “external” one [9-11] (Fig. 1), describing the locomotion in terms of deformations of the microorganism and its velocity.

These two lines of biological observations have spawned two similar trends in the modeling of amoeboids. In the literature, “internal” [12-13] and “external” [14-19] biophysical models were proposed. The latter group is of particular relevance to this work, because it represents amoeboid locomotion as a result of oscillations in the mechanical properties of the cellular structure, modeled either as a visco-elastic continuum or a discrete network, and because it shows that an asymmetry in environmental conditions that influence the characteristics of the oscillations can lead to directed motion. Moreover, in these works, the body of the amoeboid is modeled as an active hydrogel, this being a requirement for the insurgence of locomotion.

Owing to their interesting features, amoeboids have already been a source of inspiration for some works in robotics. The most notable are the evertting toroidal robots [20], and Slimebot [21-22]. Both these examples take lessons from biology, and apply them in macroscopic robots. In particular the latter is represented as a 2D network of
coupled non-linear oscillators, whose period can be related to an external stimuli (i.e. light), the gradient of which the robot climbs. Slimebot gives an experimental confirmation to the models upon which we commented in the previous paragraph [14-17] and that consider the mechanical oscillations in the cellular structure as a cause for the insurgence of locomotion. It also shows emergence of a locomoting behaviour in response to an asymmetry in the natural frequency of a network of coupled oscillators: a stimulus causes some oscillators to vibrate with a smaller period, and the coupling allows this fact to be translated into a directed locomotion through the interactions in the network. To the authors’ knowledge the design and fabrication of an amoeboid robot in a size scale comparable to that of the real organism, that is with characteristic dimension below the millimeter, has not been tackled yet.

Our aim is to investigate innovative strategies for the development of soft robots at the microscale that could in future find application in therapy and diagnostics. As part of this program, we are addressing the development of an artificial amoeboid robot by taking inspiration from the biophysical descriptions. The amoeboid microrobot should be able to move by using a source of energy available in its environment. Moreover, it should be able to follow a stimulus, for example chemical cues, to direct its locomotion. In this phase, we investigate active hydrogels as possible structural and functional materials to achieve locomotion at the microscale.

In order to address the analysis of active hydrogels for our research path we have considered the previous studies on self-oscillating hydrogels [23-24]. These materials exhibit chemomechanical oscillations driven by the Belousov-Zhabotinsky (BZ) reaction, a metal-ion catalyzed oxidation of an organic substrate by a bromated solution. In this reaction, under proper conditions, the oxidation state of the catalyst changes periodically. In suitably prepared hydrogel samples, this leads to changes in color and volume, as the different oxidation state of the catalyst moiety causes changes in hydrophilicity and thus in the hydrated volume of the material. If the catalyst chosen for the BZ reaction is an organic group functionalized with ruthenium, the color changes from orange to green, as the differently oxidized metal ion absorbs different components of incident light. The same group built hydrogel samples able to walk on a suitably prepared substrate (i.e. with ad hoc grating) by exploiting chemical asymmetries in fabrication [25], and has been working on chemical solutions to change the characteristics of the oscillation [26]. Since these materials are based on a thermosensitive polymer, exhibiting a Lower Critical Solution Temperature (LCST) above which the polymer chains tend to coil, they have an optimal temperature to maximize the amplitude of the mechanical oscillations. Since the BZ reaction is sensible to the chemical environment, we speculate that it is possible to use these oscillating hydrogels to make experimentally the structures proposed in the “external” biophysical models, and finally build a self-propelled stimuli-responsive microrobot. For example, since the period of the chemomechanical oscillation of the hydrogel decreases for higher substrate concentrations, it is possible that a properly tuned network of coupled oscillators made of this material would be able to “climb” a concentration gradient in the substrate, behaving in a manner analogous to the photo-response of Slimebot, while at the same time using the same substrate as a source of chemical energy. Our approach consists of two major steps. First we aim to implement the chemomechanical oscillators as patches of the self-oscillating hydrogel. Second we will pursue mechanical coupling by immersing the gel patches in a superstructure, for example a polymer matrix or hydrogel that doesn’t naturally oscillate; while we can expect that they would couple chemically by being in the same substrate solution. Different stimuli have been numerically shown to affect the behaviour of the hydrogels [27-28], with recent experiments confirming some of those predictions [29], thus giving different means to actually tune and control the behaviour of an artifact fabricated using this material.

In choosing this path, from biological sciences, through materials and to the design of microrobots, we neither followed a bottom-up approach, scaling up finding from nanotechnology, nor a top-down approach, resizing macro-scale devices to smaller systems. Rather, by taking a bio-inspired approach to the problem, we attempt to imitate solutions from the natural world that are already in the size range we are interested in.

In this paper, we will first describe the processes we used to realize the self-oscillating hydrogel samples and the experimental setup used for their observation; then we will proceed to compare our data with that available in the literature for the real amoeboid Physarum plasmodium and its model found in [14-15]. Finally we will draw conclusions and outline the next steps in our investigations.

II. MATERIALS AND METHODS

![Fig. 2. The chemical structure of the self-oscillating hydrogel, adapted from [23]](image)

A. Materials

Nitric acid, Sodium Bromate, Malonic Acid, Ammonium Persulfate (APS), N,N’-Methylenebis(acrylamide) (MBAA), N,N,N’,N’-Tetramethylethylenediamine (TEMED), Ethanol and Hexane (from Sigma Aldrich) were used as received. N-
Isopropylacrylamide monomer (NIPAAm) (from Sigma Aldrich) were purified by recrystallization in hexane before use. Bis(2,2'-bipyridine)-4-methyl-4'-vinyl-2,2'-bipyridine-ruthenium-bis(hexafluorophosphate) (Ru(bpy)₃) (from Cyanagen) were used as received.

B. Preparation of self-oscillating hydrogels

To synthesize the BZ oscillating hydrogel (Fig. 2), we used the recipe from [30] and modified it accordingly, in order to accommodate for the catalyst monomer Ru(bpy)₃. 100 mg of NIPAAm, 1.5 mg of MBAA as crosslinker, 1.5 mg of APS as initiator and 5 mg of Ru(bpy)₃ were thoroughly mixed in 0.5mL of bidistilled water and 0.6 mL of ethanol under a nitrogen atmosphere. The obtained pre-gel solution was put into Teflon molds and TEMED was added in 30 µL / 1 mL ratio. The gelation was allowed to completely take place in an hour. The obtained hydrogel samples were washed in a 50:50 mixture of ethanol and water for 24 hours. The washing solution was then changed to water for 48 more hours, while periodically changing the water with clean deionized one. Samples were stored in bidistilled water between uses. Thin rectangular (short side less than 1 mm) samples were cut from planar ones.

The hydrogel samples were prepared in sizes such that there would be no three-dimensional chemical wave propagating in their bulk. Hence, their thickness was less than 1 mm. Their surface area (less than 16 mm²) was chosen as a trade-off between ease of fabrication of the molds and ease of handling of the samples. Nineteen different samples were prepared for observation.

As regards the substrate for the BZ reaction, this was prepared as a solution of Malonic Acid (62.5 mM) and of sodium bromate (84 mM) in nitric acid 1M.

C. Experimental observation

Images of the oscillating hydrogel samples were obtained using a Hirox KH-7700 digital microscope (Hirox-USA, Inc.). From the output images of a continuous capture, the time-course evolution of chemical waves during oscillation was processed by a Matlab script.

III. RESULTS AND DISCUSSION

The samples showed a behavior consistent to what has been previously reported for the same materials in [31-32] both in regard to the time period of chemical oscillation (around 400 s), and to the difficulty of measuring the

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Fig. 4. Time evolution of the chemical oscillations in a thin rectangular hydrogel sample.

Fig. 5. Time evolution of the intensity of the red channel from the data used in Fig. 4.

Fig. 6. Spatio-temporal patterns of thickness oscillations in Physarum from [15]. Reprinted with permission of Springer.
amplitude and propagation of the mechanical oscillations (too small when compared to the size of the sample under the microscope).

The small entity of the observable mechanical deformation can be imputed to a number of factors, but is mostly due to the sample preparation and the experimental conditions.

The process used to make the hydrogels yields a non-porous material. Hence, the changes in hydration that would cause the swelling-deswelling happen on long time-scales, and in our samples don’t have time to complete before the chemical state changes again.

With regard to the experimental conditions, our observations were performed at room temperature. To maximize the mechanical deformations, the samples should be kept, during the oscillations, close to the LCST.

Fig. 3 (a to f) clearly shows the evolving propagation of the chemical wave in six successive instants of time (at 30 s intervals) in a typical planar sample prepared for the experiments. The figure depicts the color variations consequent to the chemical changes in the oxidation state of the metallorganic Ru(bpy)3 moiety: the green color corresponds to the oxidized state of the catalyst, while the orange corresponds to the reduced state. Moreover, the supporting movie 1 shows a time lapse video in which a full capture session is shown accelerated so that one second of video corresponds to 16 s of acquisitions.

By considering the color variations in time in a single slice of a thin (about 1 mm thick) rectangular hydrogel sample, we can observe unidirectional wave propagation and we created the plot in Fig. 4, representing the time evolution of the chemical wave in a fixed line across the sample. Fig. 5 shows the red color intensity extracted from this data: a periodic oscillatory evolution is clear.

In order to compare our data with that available in the literature for the real amoeboid Physarum plasmodium we consider [14, 15], in which Nakagaki et al. discuss a model for Physarum that places emphasis on the role of chemical oscillations as a cause for the mechanical oscillations (Fig. 6) that produce the organism’s amoeboid locomotion.

The chemical oscillator modeled is based on Ca2+ ions. Oscillators in different parts of the organism interact by the diffusion of the chemical species involved, and by transport phenomena in the protoplasm. Without the latter, the mechanical deformations that produce the movement of the organism do not exist. In this there is a strong similarity of the ectoplasm of Physarum with the materials under consideration in our investigation: as was stated above, we observe chemical oscillations, but the mechanical observations are hardly noticeable because of difficulties in the intake/outtake of water.

The period of the mechanical oscillations in Physarum is in the order of 150 s. The oscillations in this study were slower than that, the period being closer to 400 s. Faster oscillations can be obtained in more porous hydrogels, which also give the advantage of larger mechanical deformations [32] for the reasons mentioned above. Notwithstanding this difference, the behavior of the material under consideration is similar to the cellular ectoplasm described in [14] in the fact that a chemical oscillation is cause of a mechanical one.

IV. CONCLUSIONS

The present study showed similarities to the biophysical models found in the literature to explain the locomotion of amoeboids and the chemomechanical oscillations of the materials the micro-organisms are made of. Like in the organism, in the hydrogel an oscillatory chemical reaction leads to periodic mechanical deformations, which we plan to harness in creating a soft micro-robot capable of amoeboid locomotion. In such a micro-robot, the chemomechanical oscillations of different hydrogel patches should be able to couple mechanically, through the material acting as a scaffold between the patches, and chemically, through diffusion of the chemical species involved in the BZ reaction. As was shown in the literature [21-22], the coupling of oscillators that can change their characteristics in response to stimuli in the environment can allow the emergence of complex behaviors, such as directed locomotion towards a source of stimulus. With this target in mind, the next step will be to increase the amplitude of the mechanical oscillations to more easily observable levels, at the same time decreasing the period of the chemical oscillations to values allowing closer comparison with the biological inspiration.

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