BioNanoSci.
DOI 10.1007/s12668-013-0124-3

On the Loading of Slime Mold *Physarum polycephalum* with Microparticles for Unconventional Computing Application

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Abstract The plasmodium of *Physarum polycephalum* is a large single cell visible with the naked eye. The plasmodium realizes a pattern of protoplasmic veins which span sites of sources of nutrients, producing efficient network structures like cycles and Steiner minimum trees. Besides, the plasmodium can embed different chemicals; therefore, it should be possible to program the plasmodium to realize deterministic adaptive network and spatial distribution of nanoscale and microscale materials. The transported particles can be used for the modification of the physical properties of the system (electrical, optical, magnetic) facilitating the readout of the information, processed by the slime mold. Experiments with polystyrene microparticles and $MnCO_3$ microparticles demonstrate that the plasmodium of *Physarum* can propagate nanoscale objects using a number of distinct mechanisms. The results of our experiments could be employed in the field of the unconventional computing and bio-computing application devices, using *Physarum* network as scaffolds for the development of hybrid nanocircuits and microcircuits and devices.

Keywords *Physarum polycephalum* · Microparticle transportation · Unconventional computing · Adaptive network

1 Introduction

All living beings, even very simple, react to the variation of environmental conditions and respond to external stimuli. Living beings analyze the information, perform calculations in a way very different from that used by now available computers. Our work is aimed to the interfacing and implementation of simple living organisms with artificially made devices, circuits, and systems. We intend also to control their response to external stimuli in physical, chemical, and biological media and to design novel computational techniques, architectures, and working prototypes of nonlinear media-based computers. Within this research, we make steps toward the combination of biology and computer science life and computation that could find a common area of applications [1] and give an improvement in the field of unconventional computing.

The first bio-inspired models of computation are the cellular automata or cellular spaces conceived by Ulam and von Neumann in the 1940s [2]. After the early comparisons [3, 4] in the field of neural computation, between computing machines and the human nervous system, scientific community began to wonder if in the future it would be possible to develop computational techniques and systems that would yield significant computational advances by using the same criteria of how the human brain processes and stores the information, in other words, to build, at least in principle, an "intelligent computer" [5, 6].

Other approaches for the realization of the bio-inspired computational systems that are currently widely considered are based on the utilization of "memristor" [7, 8] elements, mimicking some properties of synapses [9, 10].

The life cycle of *Physarum polycephalum* (order *Physarales*, class *Myxomecetes*, subclass *Myxogastromycetidae*) consists of different stages; in the unfavorable environmental conditions (lack of sources of nutrients and humidity), *P. polycephalum* exists in a dormant stage protecting *Physarum* for long periods of time called the sclerotial stage. This condition is completely reversible, once favorable conditions resume, the main vegetative phase of *P. polycephalum*, called plasmodium, appears [11].

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Published online: 11 January 2014

The plasmodium of *P. polycephalum* is a single cell, visible with the naked eye, with many diploid nuclei. At this phase of its life cycle, *P. polycephalum* feeds on bacteria, spores, and other microbial creatures [12] englobing them in itself.

When placed in an environment with distributed sources of nutrients, the plasmodium forms a network of protoplasmic tubes connecting the food sources [12] like cycles and Steiner minimum trees.

Though it has not a real nervous system, the mold showed some form of intelligence. It has been reported that plasmodia can be used to form logic gates [13].

Nakagaki et al. [14–16] showed that the topology of the plasmodium's protoplasmic network optimizes the plasmodiums harvesting on the distributed sources of nutrients [12]. The plasmodium is capable for an approximation of shortest path, computation of planar proximity graphs and plane tessellations, primitive memory, and decision making. The plasmodium is considered as a parallel computing substrate, complementary [17] to existing massive parallel reaction–diffusion chemical processors [18].

As *Physarum* reacts in a consistent way to stimuli (light and food sources), it can be considered as the "ideal substrate for future and emerging bio-computing devices" [19].

For the development of self-growing computational systems, we must consider and study the slime mold's potential of integration and redistribution of foreign particles.

It will allow us to use *P. polycephalum* as a programmable transport medium as showed by previous attempts to mix substances, for example dyes [12] and microparticles [20, 21] within *Physarum.*

Here, we present our first experiments on the loading of slime mold with inert microparticles of two types: polystyrene and manganese carbonate ($MnCO_3$). The latter is soluble in a certain condition (acid or salt solution treatment). Our purpose was to highlight the *Physarum* compatibility and ability to transport microobjects of different nature and, if the electrolyte species of such living species can, in some way, modify the materials that are in contact with it.

2 Methods

2.1 Culture and Microparticle Intake

Plasmodium of *P. polycephalum* is cultivated in plastic containers and on paper towels sprinkled with distilled water and fed with oat flakes (Asda's Smart Price Porridge Oats, UK). Experimental supports for the electron microscope imaging were aluminum stubs or silicon substrates. In each experiment, a piece of plasmodium is placed in the center of the stub. The experiments of mold growth on conductive supports (aluminum, silicon) were done using a hermetically closed chamber by raising the stubs from the bottom of the chamber, filled to 2/3 with a saline (K_2SO_4) solution at 95 % which provides moisture conditions, necessary for the survival of the mold. We use two types of nanoparticles:

- Polystyrene microparticles (Sigma-Aldrich) consisting of an aqueous dispersion (% 0.5 p/p) of particles with a diameter of 3 μm (Fig. 1A).
- $MnCO_3$ microparticles (PlasmaChem GmbH, Berlin) with a diameter of 3 μm in an aqueous dispersion (% 0.5 p/p) (Fig. 1B).

The first particle solutions were applied directly onto the plasmodium, typical application volume was 20–30 μL, mechanically mixing the plasmodium with microparticle solution for about 1 min to allow the plasmodium to absorb the solution well, the excess unabsorbed solution was eliminated after the plasmodium treated with microparticles was placed on silicon. On silicon, in front of the mold (about) a flake oatmeal was placed to realize the formation of a vein on substrate.

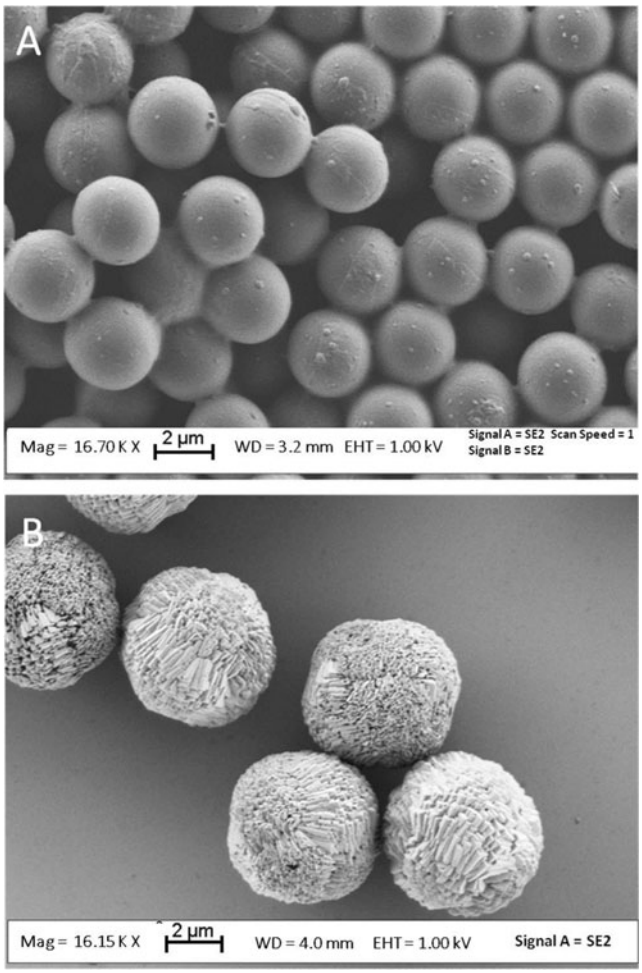

Fig. 1 Scanning electron microscope (SEM) images showing **A** polystyrene spheres with 3 μm diameter and **B** $MnCO_3$ microparticles with 4 μm diameter

To facilitate directional growth of the plasmodial tree, we placed oat flakes (food) near the edges of the stub, about 2 cm from the slime mold mixed with polystyrene microparticle inoculation zone. The plasmodium on the samples was flowed in the stage of sclerotia with white light and drying before being mounted in scanning electron microscopy (protoplasmic networks about 1–1.5 cm away from the inoculation site are used in scanning electron microscopy studies). SEM measurements were done with Microscope SEM Zeiss Supra 40 with the acceleration voltage of 1 kV.

3 Results and Discussions

3.1 SEM Studies of *P. polycephalum* in Sclerotic Stage

First of all, we studied the morphology of *Physarum* in sclerotial stage by SEM analysis.

Figure 2A shows *Physarum* pseudopodia formed during the process of motion towards nutrient sources, while Fig. 2B shows a *Physarum* vein. Using the detector placed outside the acceleration column (SE2), it is possible to highlight the lumpy matter surrounding the plasmodial vein, it is, probably, the metabolic residues of the mold on the aluminum (Fig. 2B). During the induction of the *Physarum* sclerotial stage, by drying, it is possible to see the formation of multiple cracks in the *Physarum* body and tubes (Fig. 2B). Figure 2C shows *Physarum*'s body section "sponge-like" in one of these cracks.

3.2 Microparticle Intake

Slime mold, treated with microparticle suspension, was noted to not only survive but also to preserve its natural behavior, such as the ability to move and to located food sources. Figure 3A shows dry tubes which were rather far (about 1 cm) from the slime mold mixed with polystyrene microparticle inoculation zone. There is a considerable amount of particles transported along the network of veins on the outside of the protoplasmic membrane.

It is interesting to note that there are different mechanisms of particle transport.

The particles can be transported on *Physarum* vein surface exposed to external environment, but they can also be taken inside the plasmodium.

Figure 4 shows the presence of polystyrene particles within the crack inside the *Physarum*'s body, formed during the drying; it appears evident that the *Physarum* is able to incorporate and integrate microobjects.

Figure 5 shows the $MnCO_3$ microparticle transported by the slime mold on the vein without integration (Fig. 5A), and,

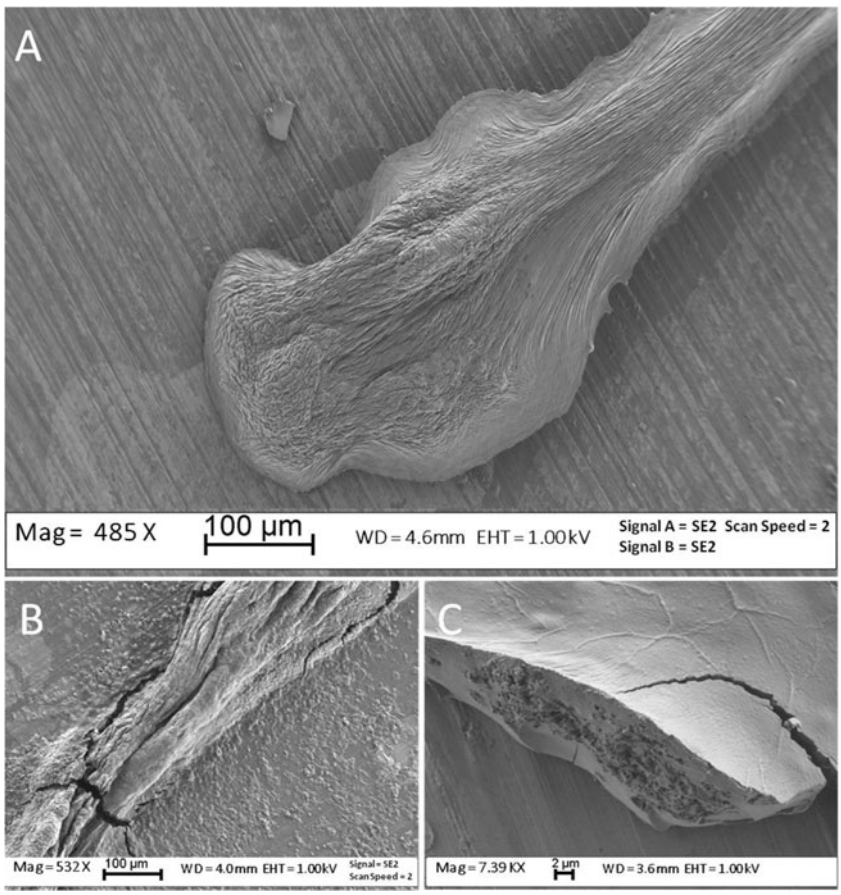

Fig. 2 SEM images of *Physarum* in its sclerotial phase on aluminum stub

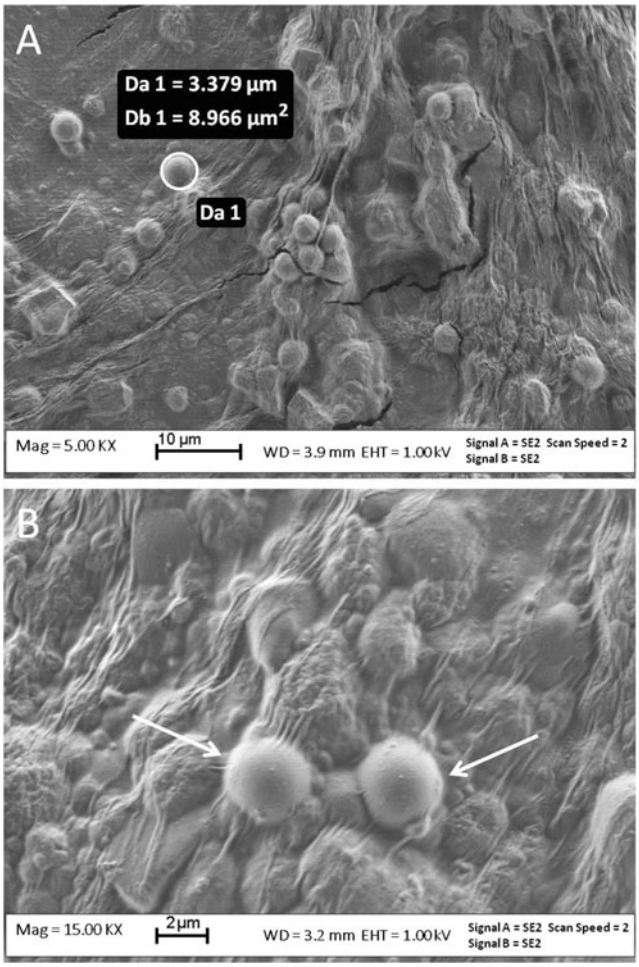

Fig. 3 SEM images of protoplasmic tubes from plasmodium treated with polystyrene microparticle suspension

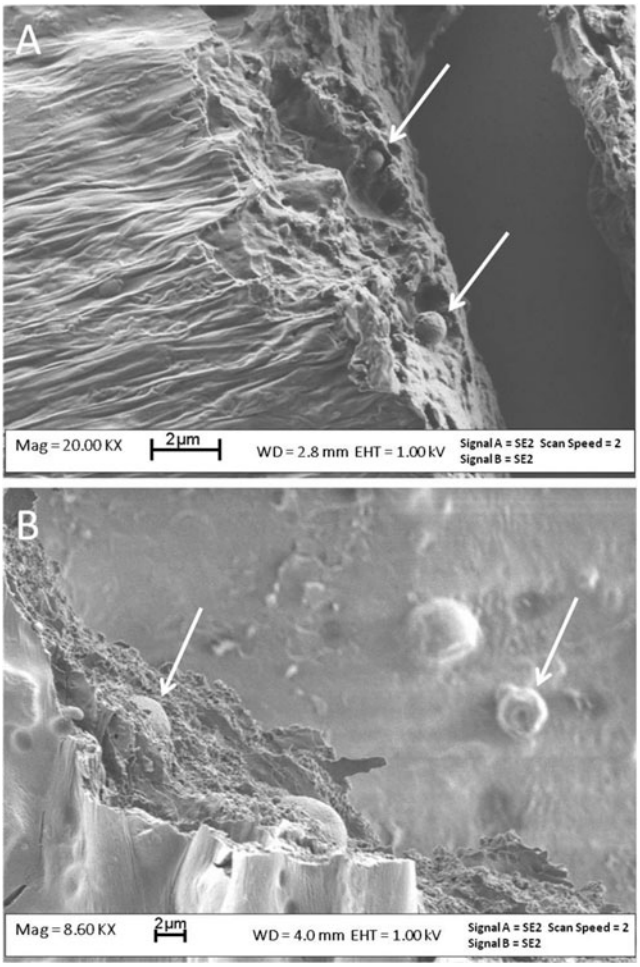

Fig. 4 SEM images of the sections of protoplasmic tube from plasmodium treated with polystyrene microparticle aqueous suspension

inside a protoplasmic tube (Fig. 5B), both images were obtained with the same detector (SE2) on tubes which were rather far away from the zone where the $MnCO_3$ microparticle solution was applied. $MnCO_3$ microparticles are usually in crystallized form and have a rough surface, as it is shown in Fig. 1B. In Fig. 1B is shown $MnCO_3$ microparticles initial morphology; mixing $MnCO_3$ microparticles with the slime mold occurs that when the particles are on the surface of the slime mold, they retain their initial morphology, as it is shown in Fig. 5A, while the particles are inside the body of the slime mold, and what happens to the majority of the particles, have rounded profiles and a very smooth surface, as it is shown in Fig. 5B.

When particles integrated in *Physarum* are inert, such as in the case of polystyrene microparticles, they, as it should be, appear unaltered within the sections of protoplasmic tube from plasmodium.

Instead, when the mentioned inorganic particles are inside the *Physarum*, the internal electrolyte can provide some etching of $MnCO_3$ microparticle surface, smoothing it. The conspicuous presence of $MnCO_3$ particles, smoothed and polished rather than crystallized, can be considered as a further demonstration of the *Physarum* ability to incorporate and integrate microobjects into its body and to carry them across the network of protoplasmatic veins.

4 Conclusions

As a result, we can conclude that the *Physarum* can be loaded with microparticles and nanoparticles of different materials (inert organic and inorganic) without losing the mobility and its physiological activity. We have demonstrated, according to the previous studies [20, 21], that the plasmodium of *P. polycephalum* can transport the nanoscale objects during its propagation and that the slime mold is capable of the integration and redistribution of foreign particles, that can be useful for the development of the novel techniques and hybrid nanocircuits and microcircuits or devices in which it is necessary to create, for example, a map of microelectrodes and nanoelectrodes with different nature (silver nanorods, gold nanoparticles, different magnetic materials, etc.). The

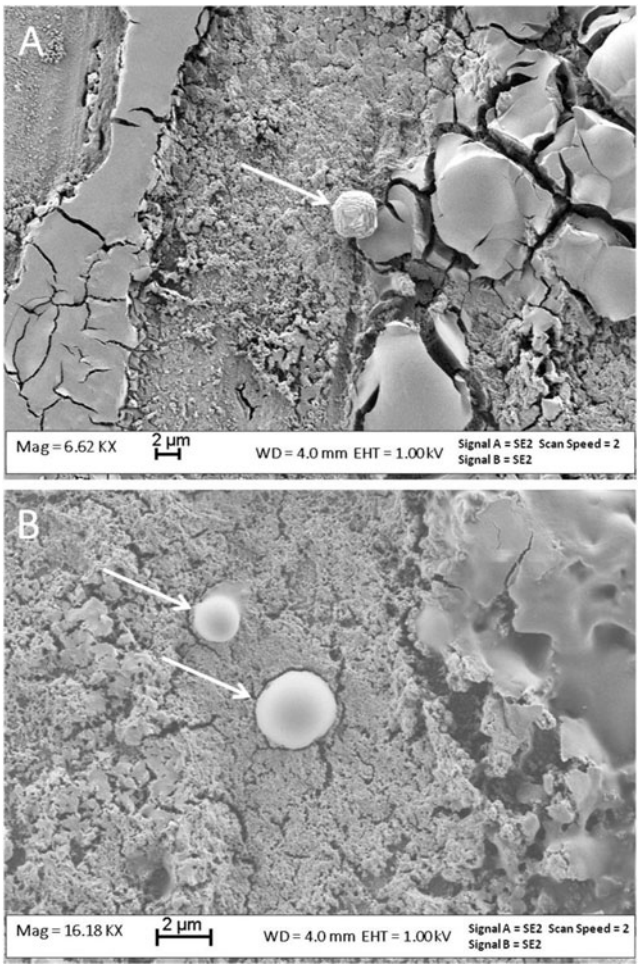

Fig. 5 SEM images of protoplasmic tube from plasmodium treated with $MnCO_3$ microparticle suspension

obtained results allow us also to suggest that other types of microobjects and nanoobjects can be incorporated into the slim mold. In this respect, nanoengineered polymeric capsules (NPCs) [22] can be of great interest. NPCs can be loaded by different substances, and the encapsulated molecules can be successively released in adequate environmental conditions or by applying the external action [23]. In particular, it is possible to load specially prepared NPCs with substances, varying the metabolism of the slime mold, and to perform the release with their illumination [24] with green light. Such an action will provide additional possibilities of the information processing when the external action will be superimposed on the internal activity of the slime mold.

Acknowledgments We acknowledge the financial support of the Future and Emerging Technologies (FET) program within the Seventh Framework Programme for Research of the European Commission, under the Collaborative project PhyChip, grant agreement number 316366.

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