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GROWTH OF TEMPLATED GOLD MICROWIRES BY SELF ORGANIZATION OF COLLOIDS ON *ASPERGILLUS NIGER*

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Template assisted self-organization of inorganic nanoparticles was investigated to render the physicochemical properties of both nanoparticles and biological materials in hierarchical architecture by using chloroauric acid and Ajinomoto® (mono-sodium glutamate, MSG) that served the dual purpose of stabilizing the NPs in the gold colloid and also nutritional source for growth of *Aspergillus niger* (*A. niger*) hyphae and mycelia (acting as living template). The coating of gold nanoparticles on living hyphae was controlled by varying MSG concentration to gold salt and the reaction temperature without requiring any hazardous re-agent. Grown microwires displayed wide variations in dimension and morphology depending upon the preheating and nutrient conditions. Uniform and thick agglomeration of gold nanoparticles at higher molar ratios (MR's ~ 10 and 12) formed microwire of diameters between 1-2 μm and length exceeding 1 mm within two weeks. Heat treatment above 40-45^{0}C led to negligible growth, wide variation in diameter (1.1-3.6 μm) and significant reduction of gold colloids due to excessive surface evaporation, whereas, maximum morphological changes in microwires were observed at 30^{0}C, having diameter 2.1-2.9 μm. The pH of the gold colloids was found to change gradually from 3 to > 7 during the growth process indicating the successive aggregation of gold nanoparticles on living hyphae and the consumption of glutamic acid by the microbes. High surface area of these bio-templated gold microwires is interesting for sensing, electronics, optics and catalysis applications.

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Keywords: Self-organization, Gold microwire, Colloid, Microbial template, *Aspergillus niger*

1. Introduction

Template based engineering through self-organization of metal nanoparticles is becoming more promising for the fabrication of low cost and highly efficient functional micro-scaled structures. Unique morphology of micro-organisms like virus [1, 2], bacteria [3], diatoms[4], yeast [5], bacteriophages [6] and fungi [7] have been used by many researchers as templates to build functional hierarchical structures that have potential applications in optics [8, 9], photonics [10] electronics [11], catalysis [12], magnetism [13, 14] and in biotechnology [15]. For example, the use of live bacterium as template to build conductive hybrid system [16] and gold decorated spider silk for efficient vapour sensing [17] have been reported.

Colloidal self-assembly and self-organization are simple fabrication techniques to build highly ordered and precise nanostructures with minimal energy requirements[18].

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Further, the molecular interactions of colloidal nanoparticles are based on naturally driven forces like hydrophobic verses hydrophilic components, entropy, gravitational, van der waals or columbic interactions [19, 20].The tuning of nanomaterial properties fabricated by templating, including surface area, electrical, optical and mechanical properties depend upon the morphology of the nanostructures. For example, one-dimensional nanotubes [21, 22] nanowires [23-26] super lattice structures [27], two-dimensional and three-dimensional assemblies [28] instead of nanoparticulate films [29, 30] lead to higher surface to volume ratios**.**

A simple and facile chemical method to promote self-organization of gold nanoparticles on growing mycelia of *A. niger* to fabricate bio-assembled gold microwires without requiring any complex functionalization has been reported earlier as preliminary report by our group that lead to highly conductive (2.5×10^{-8} Ω m) gold microwires typically 1-2 micrometers in diameter and length exceeding few millimeters[31].

In the present work we have optimized the growth of these microwires by using the same precursors, chloro auric acid and MSG (mono-sodium glutamate was used as reducing and stabilizing agent to synthesize gold nanoparticles [32] and also serving as nutritional trigger behind the self- organization of gold nanoparticles on *A. niger* hyphae served as a living template to self-organise the nanoparticles)[31] but controlled different parameters such as preheat treatments and nutrient concentration gradient (various concentration of MSG to fixed amount of gold salt) in order to display wide variations in dimensions (diameter ranging from 1.1-3.6 μm) and morphology of microwires to get an insight to the best growth conditions. Various experiments revealed the fact that faster and thicker microwires could be grown at preferably 30^0C. Contrary to what we had expected we found that the growth rate increases with decreasing glutamate contents but non-uniform attachment of gold nanoparticles were observed which were highly resistive. Whereas microwires fabricated with gold colloids and glutamate content equal to or slightly higher than the auric acid (MR ~ 10 and 12) are best suited for the growth of uniform mesh of wires. Variations in pH values and visible absorption spectrum have been studied to clarify the mechanisms and interactive forces involved in this bio-composite assembly of gold nanoparticles on growing hyphae. These extensive studies can lead to a controlled use of various bio-templates by tailoring their growth and hence properties, under constrained external factors towards achieving different bio-composite micro and nano scale hybrid structures for varying applications in electronics and optics.

2. Experimental

Gold nanoparticles were obtained by the reduction of chloroauric acid (Sigma-Aldrich) with glutamic acid (Sigma-Aldrich) in aqueous media as reported earlier[32-34]. Briefly, 2 ml of 5mM gold chloroaurate ($HAuCl_4.3H_2O$) solution was diluted by 50 ml deionized water (>18 MΩ cm^{-1}) and the mixture was heated until boiling. In order to investigate the trend of microwire growth under nutrient stress, different colloids with different molarities were prepared by varying glutamate contents and keeping the concentration of chloroauric acid constant. Aqueous solution of 25 mM monosodium glutamate (with molar concentration of 3,5,7,10 and 12) were added in the boiling solution under continued heating until the colour changed from mild yellow to bright red, normally within 5-10 minutes, indicating the formation of gold nanoparticles. When there was no further change in colour of colloid the solution was rapidly quenched in an ice bath to arrest the growth of nanoparticles.

Fungal strains of *A. niger* (TISTR 3013), from phylum Ascomycota, were cultured on potato dextrose agar (PDA, Difco Laboratories) medium at 27- 30°C for 3 days by using flame streak method [35].The fungal culture used in this work was obtained from Thailand Institute of Scientific and Technological Research Bangkok, Thailand.

Fungal colonies were scraped and mixed with 0.5 ml sterile water (autoclaved deionised water) in eppendorf tubes by using vortex mixer for ~1 min to break the conidia chains and to separate out conidia from mycelia. The solution was filtered through sterile cotton wool, to remove

other residues, centrifuged and washed in de-ionized water. Adjusted conidia suspension of 6×10^6 conidia /ml (measured by Petroff-Hausser counting chamber) was used for all the experiments. As prepared conidia suspension of *A. niger* (pre-incubated for 15-20 hrs at 27 ± 3^0C for spores' to achieve stability for suitable hyphae growth) was added to 50 ml of glutamate capped gold colloids in order to allow fungal growth. The entire set up was placed in incubator at temperature of 30 ± 2^0C and 80% rH. The experimental set up for the growth of microwires is schematically represented in Fig. 1.

Optical absorption spectra of gold colloids were measured by using USB 4000-FLG diode array spectrophotometer (Ocean optics) within UV-visible wavelength range of 200-1100 nm. The electrical measurements of these microwires have been performed by measuring the resistance with KEITHLEY 618 programmable electrometer.

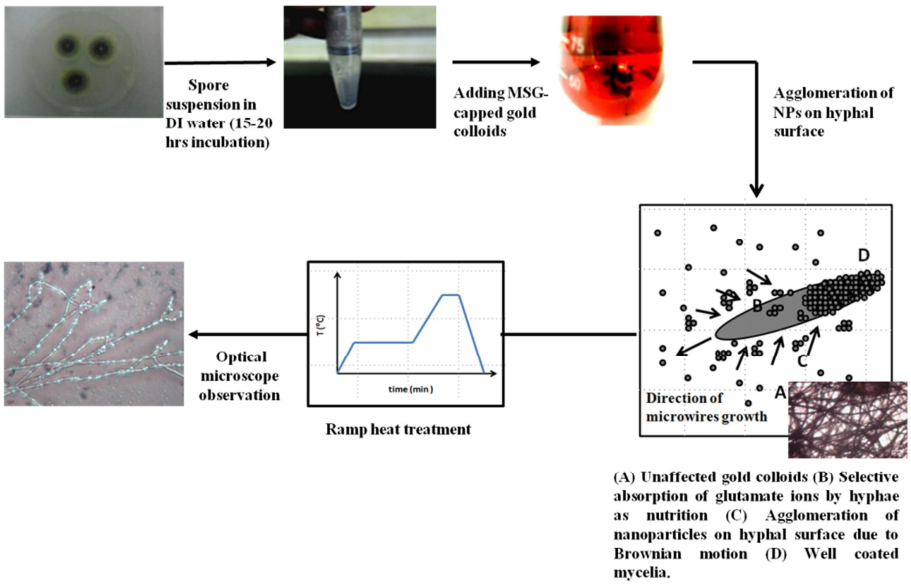

Fig. 1. Schematic illustration of biologically templated growing microwires based on self-organization of glutamate-capped gold colloids on A. niger hyphae.

3. Results and discussion

Systematic observation of growing hyphae under microscope within the first week of growth revealed that *A. niger* spores absorbed nutrients from glutamate ions, acting as a source of carbon and nitrogen through partial oxidation into ammonia and carbon di-oxide, essential for fungal growth[36-38]. The removal of glutamate ions from the colloid in the vicinity of the fungal walls de-stabilized the charged nanoparticles and consequently the metallic nanoparticles aggregated on growing hyphae by following the geometric confinement provided by the fungal cell wall that acts as template and thus formed highly stable bio-conjugated hierarchical structures.

3.1. Self-organization of gold nanoparticles on living hyphae

UV-visible absorption spectra showed reduction in absorbance peak of gold colloids (for MR's of 3, 5,7,10 and 12) after exposure to growing hyphae for period of time as shown in figure 4. This reduction in optical absorbance peak was due to the loss of gold nanoparticles from the colloidal solution due to the agglomeration onto the fungal walls. For lower concentrations of glutamate in the colloids (MR's ~ 3, 5 and 7), reduction in absorbance peak was faster probably due to rapid consumption of glutamate ions caused by the faster growth and germination of hyphae for pH ~ 4-4.5 [39]. Hence, faster de-stabilization of gold colloids occurred and the nanoparticles agglomerate non-uniformly on the fungal wall (figure 2 (a)). On the contrary, when higher

concentration of glutamate ions were available in the colloid (MR's of 7, 10 and 12) a gradual reduction in absorbance peak was observed and a uniform and even distribution of de-stabilized AuNPs on *A. niger* hyphae could be observed (figure 2(b)).

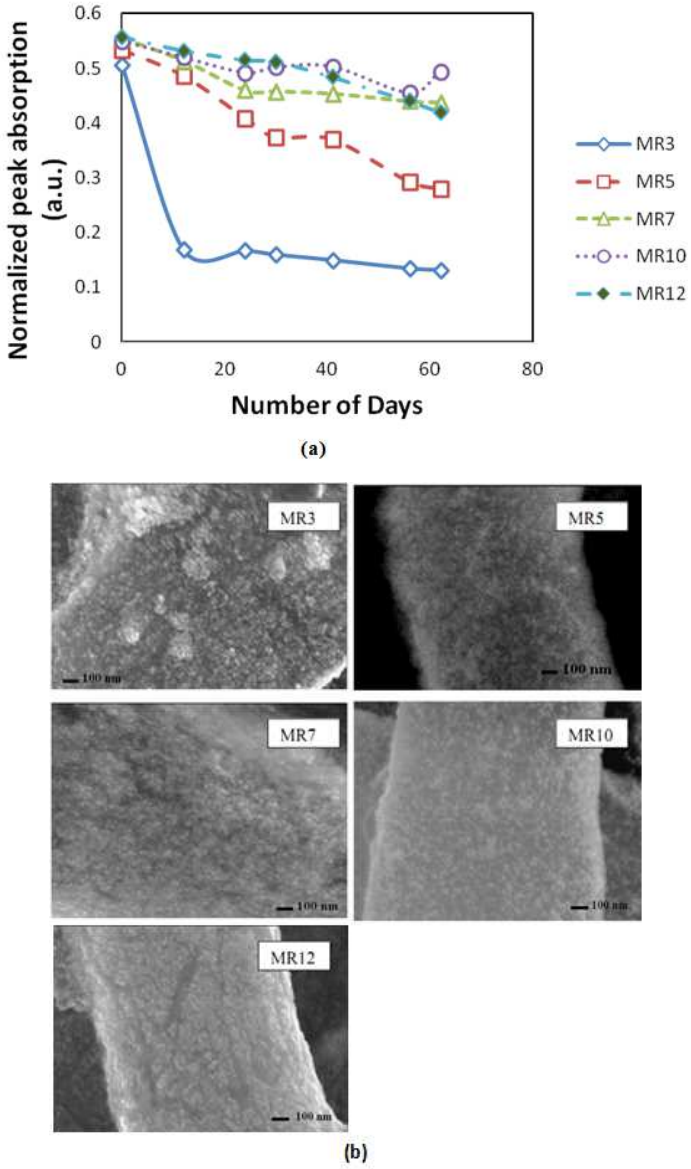

Fig. 2. (a) Change in the absorption peak of gold colloids in terms of growth periods of gold microwires (b) SEM images for A. niger templated gold microwires without light source for different molar ratios.

Concentration gradient and entropic contribution may play a role in polarizing the de-stabilized gold nanoparticles to stay on hyphal wall as long as excess of glutamate ions keep on decreasing around the fungal hyphae upon continuous fungal growth. Thermodynamic free energy of adhesion may also be involved in keeping the observed cylindrical assembly [40]. The accumulation of AuNP's on fungal cell wall can be ascribed to Brownian motion that force the nanoparticles to shift and assemble towards growing *A. niger* hyphae and thus nanoparticles organized themselves into subsequent layers. The interactive forces between the nanoparticles hold the assembled structure stable and robust without any extra energy or other adhesive forces. As gold and hyphal surfaces are hydrophobic, so this assembly of AuNPs onto fungal hyphae can be attributed to interactions between two hydrophobic surfaces, dependent on the interfacial free energy between liquid and surfaces [41, 42].

pH variations of gold colloids after fungal exposure inferred that pH follow the trends towards basic region (pH ~ 3 to > 7 at all MR's) instead of acidic (decreasing pH values) during the growth of microwires (figure 3) due to consumption of glutamate (having pH ~ 6 in solution form) by the growing fungi. This shifting in pH may be ascribed to breaking of large molecules of mono-sodium glutamate (MSG) into smaller molecules like amino acids (specifically GABA gamma amino butyric acid as well as leucine, isoleucine, valine, and methi-onine [43, 44], that served as nutrients for *A. niger*. It has been demonstrated that glutamate alone is sufficient source of nitrogen for *A. nidulans* due to suppress enzymes that are involved in catabolism of nitrogen [38]. These amines, further break into smaller molecules to be served as nutrients by fungi like carbon (C) and hydroxyl (OH^-) ions from carboxylic acid group, nitrogen (N) by assimilation of ammonium ions (NH_4^+) , hydrogen (H) and sodium ions (Na^+). The opposite ions combine in the aqueous media to form sodium hydroxide NaOH and thus the colloidal solution turns basic during the self-organization process[45, 46]. The pH of the growth solution stabilized upon reaching at pH ~ 7.0 which indicated the saturation in microwires growth. Upon drying, these metallic microwires showed yellow lustre indicating the optical and electrical properties close to bulk gold [47].

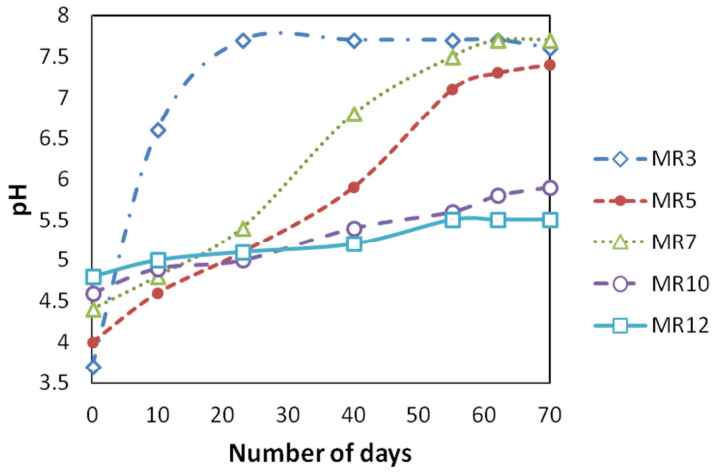

Fig. 3: Change in the pH values of gold colloids against growth periods in terms of number of days for different molar ratios between glutamate and gold colloids following the trend of basicity indicating many morphological changes within fungal growth like increase in surface area to volume ratio.

Table I. Change in pH values of gold colloids at various molar ratios (MR's) after fungal exposure.

Days	MR3	MR 5	MR7	MR 10	MR12
0	3.7	4.0	4.4	4.6	4.8
10	6.6	4.6	4.8	4.9	5.0
25	7.7	5.1	5.4	5.0	5.1
40	7.7	5.9	6.8	5.4	5.2
55	7.7	7.1	7.5	5.6	5.5
62	7.6	7.3	7.7	5.8	5.5
70	7.5	7.4	7.7	5.9	5.5
150	7.8	7.7	7.9	7.5	7.3
170	7.9	7.75	7.9	7.5	7.4

3.2. Growth of wires at various temperatures

Effect of temperature on fungal growth was studied by growing microwires at four different temperatures, viz. 25^0C, 30^0C, 40^0C and 45^0C. The optical absorption spectra of fresh colloids and after 15 days exposure to *A. niger* (for colloids formed at constant molar ratios) at different temperatures (figure 4) showed successive agglomeration of gold nanoparticles on to the fungal walls. No changes in the colloids volume were observed at both the studied temperatures 25^0C and 30^0C, but reduction in absorbance peak was much higher at 30^0C that could be due to the faster growth of A. niger at this temperature [39] leading to the consumption of glutamate and the subsequent loss of gold nanoparticles from the colloids onto the fungi. The optical absorption peak was found to reduce from 0.5 to 0.409. In contrast, higher optical absorption peak (approximately 0.162) was recorded when the growth was at temperatures above 40^0C, which was found predominantly to arise from the significant volume reduction of colloids due to excessive surface evaporation. Thick microwires with maximum morphological progression having diameter within the range of 2.1 μm to 2.9 μm were observed at 30^0C (Inset: figure 4).

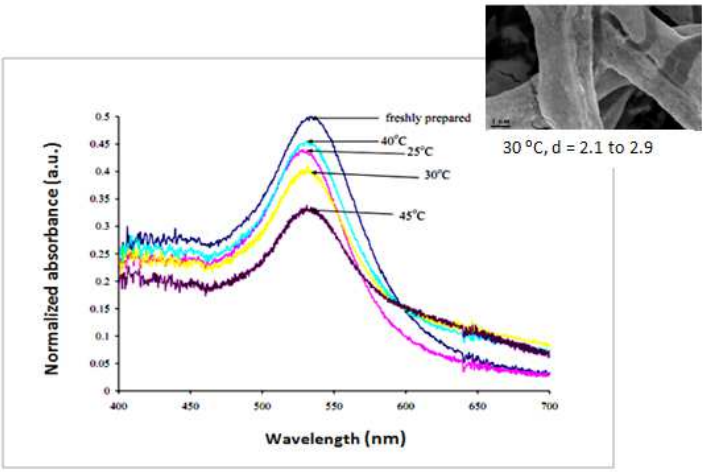

Fig. 4. Visible light absorption of the glutamate capped gold colloid with fungal growth of 15 days kept at different temperature viz. 25 ºC, 30 ºC, 40 ºC and 45 ºC Inset: SEM image of A. niger templated gold microwires at optimized temperature 30 ºC with measured diameter 2.1-2.9 μm.

3.3. I-V Characteristics

Electrical characteristics of the gold microwires were studied by pre-drying at room temperature for 1-2 days to remove moisture. In order to preserve the 3D structure of these fabricated microwires, ramp heat treatment was carried out over a period of 13 hours [48]. First the samples were heated from ambient temperature (room temperature ~ 25°C) to 100°C at the rate of 1°C/min to evaporate all inorganic residues slowly and then allowed to stay at 100°C for 5 hrs. Thereafter the samples were heated slowly to a temperature of 300°C (~ 1°C/min rate). This gradual heating was necessary as nanoparticles can maintain mesh of wire instead of forming thin films by collapsing. The resistance of these gold microwires was found to be ~ 50 Ω /mm as confirmed from measured I-V characteristics showing Ohmic behavior with a linear increase in current at applied voltage (figure 5). The measured resistance is low enough to confirm the conductivity of these microwires.

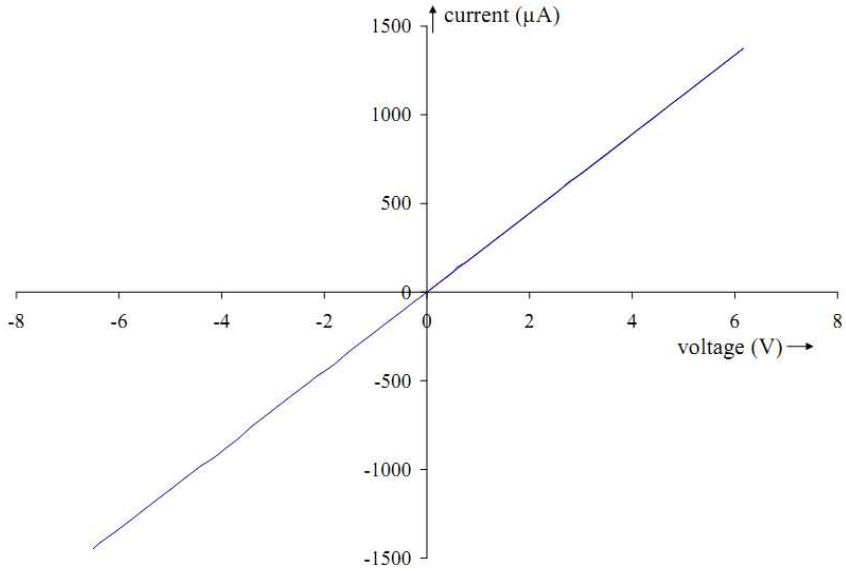

Fig. 5: Current-voltage characteristics of a typical gold microwire showing Ohmic characteristics after ramp heat treatment over a period of 13 hrs

4. Conclusions

In summary, this work highlights successive control on morphology and metabolic pathway of *A. niger* to fabricate biomimetic microwires through self-organization of glutamate capped gold nanoparticles on living hyphae as template. Significant agglomeration of AuNP's was achieved by optimizing various growth parameters as nutritional contents (glutamate to gold salt concentration), humidity and heating treatment. Detailed experimental studies reveal that increasing growth rates of fungi at lower molar ratios (less glutamate content) limits the growth of fungi and thus lead to the formation of weaker wire meshes due to non-uniform coating of AuNP's on living fungal substrate. For the growth at 30^0C with higher MSG contents fast and uniformly coated gold microwires (diameter ~ 1-2 μm and length beyond 1mm with uniform and thick coating of AuNP's) were obtained. Gold nanoparticles self-organize on the hyphal surface mainly due to the nutritional trigger (glutamate consumption) while entropic disorder, hydrodynamic forces, Brownian motion as well as hydrophobic interaction between fungal and gold surfaces assist in the complex hybrid self-assembly. Thus the assembly of nanomaterials within bimolecular components provides robust, structural and functional diversity in complex physical confinement. This theme of template engineering can be generalized to the variety of in-organic nanoparticles to form versatile and multifunctional structures by following the inherent geometry of living biological entities.

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