EXTENDED ABSTRACT

Polymer and Biological Nanowires
The Preliminary Program for 2005 Annual Meeting (Cincinnati, OH)

Genetic Engineering of Escherichia Coli K-12 for the Development of Nano-tubes (fimbriae=pili) for Effective Drug Design,# 138191

Nitosh Kumar Prof. Brahma,
Chemical Engineering, Department of Chemical Engineering,
Indian Institute of Technology, Kharagpur-721302, India,
Kharagpur-721302,, Kharagpur-721302,

One of the major breakthrough at the present scientific and engineering world would be the development of specific molecular structure oriented drug and enzyme –designs as bio-nano-tubes. However their techniques are very laborious and costly. The microtubules of E.coli has been recently found to be very active to pursue such drug delivery. These protein appendages were the elongated surface appendages (fimbriae=pili) of Escherichia coli and other microbial species, Fig.1. They are specific and are used particularly to stimulate immune response and cell lyses and removals of toxic soil contaminants (aromatics=phenols) in the process of biotransformations or by bioremediations. A case study causing diarrhea to Balb/c mice was considered to validate the process, specificity to protect mice against 026:EPEC (enteropathogenic Escherichia coli) fatal diarrhea. Genetically engineered E.coli K-12 was made for these specific drug and immune response and for removals of toxic ingredients from the soil. In the present paper the author will discuss the method in which the specific drug application, specially the immune response based micro-nano-tubes (fimbriae=pili) can be developed through which the drug applications could be faster.

Nano in Greece is small. “Aladdin wonder fictitious Lamp “ may comes as true, that after friction of lamp, the particles release such an amount of energy, that it can solve all desirable problems. The energy concept of ***Einstein*** $E= mc^2$ and ***Max-Planck*** $E= h.\nu$, ***Debye-Hueckel, Log*** $y = -[z+, z-] AI^{½,}$, and thermodynamically the energy concept of ***Entropy*** $dS= dQ/T$ perhaps would show the relationships and the measure for orderly situation of molecules, if the dealing of molecules are made at the range of 10^{-9} meter(m) nano-particle(NP) However NP must be benign to increase the life span of instruments, materials, the human system, to prevent and loss of energy and pollutions. A few years ago, during eighties several elemental metallic and later organo-metallic complexes were searched to develop, this film and super conducting materials. In the recent time NNI (National Nano-Initiative) constituted the future of objective of Nano-technology. Four generations of Nano-technology have been proposed. In (2001-) namely; 1^{st} generation, has been considered as passive nano-structure. Primary research was focused on Nano-structure, materials and tools. In the 2^{nd} generation, since from the year (2005-) it has been focused on active Nano-structure,

including Nano-bio-sensors. In the 3rd generation, starting from (2010-) onwards, the research objective was focused on 3 –D Nano-systems and the use of total "Nano-system (NS)". And starting from 4th (2015-) generation the Nano-system was focused on heterogeneous design of molecular nano-systems. In this paper the author will discuss the innovative and evolutionary concept of Nano-technology (NT) with biological constituents, to prove, that the negative side of nano-particles (NP) as health and environmental hazard can be prevented, if NPs are being made with help of microbial system.

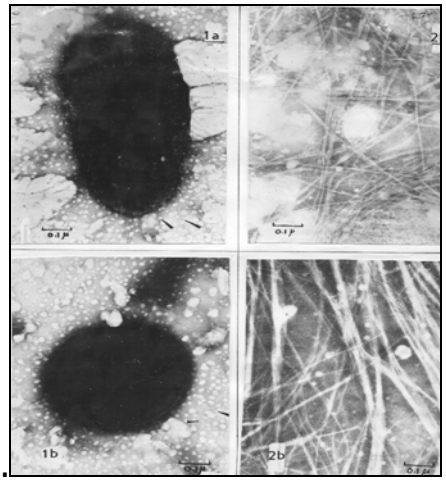
Fig.1. 

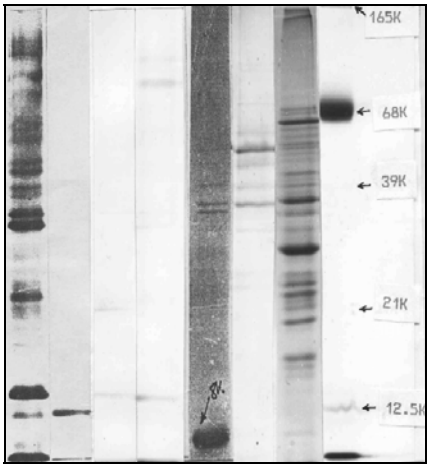
Fig.2. 

Fig.1. the surface antigenic properties (elongated protein appendages), bio-nanotubes, microtubules=(filbriae=pili) have been projected. The length of 1cm= 1 micron(μ) and similarly the diameter and length of fimbriae=pili=bi-onanotubes can be measured. Each of these elongated fimbriae=pili (bionanotubes) are specific to individual microbes and can play specific drug design and the infections to mammalian bodies, by means of colonization and transmitting endo- and entero-totoxins.. The hybrid protein appendages can be made for specific immune system, in which the main features of adherence and colonization of microbes were searched and the peritoneal macrophages were trained to identify the similar appendages and to kill microbes, which might have carried the infection. The development of such transgenic hybrid, genetically engineered E.coli K-12, C600 Yale auxotrophic strains were made by transfer of extra chromosomal plasmid-DNA of the donors, who are identified causing diarrhea, and supports tumor growths. Data reveled that the specificity to prevent infection (diarrhea, drug release tumor growths inhibition) or biodegradations = biotransformation of soil can be enhanced through this unique technique as cost-effective ecofriendly applications. For the production of specific drug designed molecules (nano-molecules, nano-tubes) the applications of genetically engineered bacterial methods would be essentially as challenging attempt. Fig. 2. The SDS (Sodium Dodecyl Sulphate polyacryl amide gel electrophoresis) was made to perform the nature of proteins, isolated as nano-biotubes, and remained either as conjugated molecules or as fragmented molecules. Nano-bio-tubes (fimbriae= pili) SDS and agaraose gelelctrophoresis follow the micro technology and MEMS.(microelectronics mechanical system) covering microfuiding activities of cells and matrix at high potential difference(voltage) and milli ampere (mAmp)current =flow activities.

NNI (National Nano-technology Initiative transforming plan proposed in 1999 led to synergistic, accelerated and interdisciplinary development. As already mentioned the global challenges to compensate the applications of " nano-Technology" namely in " Energy loss, potential drug applications and for sustainable engineering applications. Four generations of Nano-technology (-2001) namely; 1st generation, passive nano-structure. Primary research was focused on nano-structure, materials and tools. 2nd (- 2005) generation was focused on active nano-structure, including nano-biosensors. 3rd generation (-2010) 3 –D nano-systems and system of Nano-system. And 4th (2015) generation was focused on heterogeneous molecular nan0-sytems. ***Echerichia coli K-12 C600*** Yale strain is considered to be the most favorable dynamic bioengineering tool, for designing vaccine, nano-particles, nano-tubes and several other composite and bio-transforming chemical products.

Preparation of Biological nanotubes (BNT).

Identify the cell (microbe)→ Search their DNA and plasmid properties→ Identify cell→(Hybrid or wild types)→ study DNA propertie, plasmid in Agaose gel→ Specify its activities by DNA analysis.→ Grow the cells in selective sterile medium→ Isolate the cell and dissolve them in Tris.HCL, pH 7.8 buffer→ Homogenized or shear the cells by specific stirrer→ centrifuge the homogenized cells at 10,000 rpm→ Separate and decant the supernatant→ Into a clean and sterile centrifuge tubes→ centrifuge the supernatant in 12-15,000 rpm→Decant the supernatant to a very clean 100 ml sterile conical flask→ Add ammonium sulphate ($NH_4)_2 SO_4$ till saturation was reached at cold(4 ^{o}- 10 ^{o}C), overnight (12 hours) by continuous slow stirring as made by magnet stirrer→ CEntrifuge the precipitate at 20-25, 000 rpm→ and decant the supernatant→ Dissolve the precipitate (ppt) in sterile and in clean Tris.HCl buffer at pH 7.8.→ Use the isolated hybrid E.coli, the isolated SA (surface antigens) pili=fimbriae= nano-tubes, in animal test. In this case the preliminary Balb/c mice and later domestic animal tests were performed.The isolated Nano-tubes are being characterized found to be different by their length, diameter & compact natures→SDS-PAGE shows established that they are also different in their protein contents.

Scientific American's ***UNDERSTANDING NANOTECHNOLOGY*** represented this technology as the cutting edge technology that will find usage in medicine, space exploration, communications, manufacturing, and almost every other aspect of modern society. Imagine getting an injection of "smart" molecules that can seek out cancer cells and destroy them without harming any of the surrounding tissue. Imagine a simultaneous space launch via the Shuttle of thousands of robotic probes, each no bigger than an insect, and each programmed to do a single task in concert with all of the others. And that's just the beginning. Finally few structures of microchips and the measuring devises have been recapitulate, to establish the relations of ***Einstein and Max-Planck*** energy concept and to understand the relationships of light velocity and frequency, the concepts of photons and quantum, which will support our understanding both biological and material engineering in future. So it might be realistic to say, that A. Einstein is also a nano-particle, if he is compared to his vast potential theory of relativity and quantum mechanics, $E= mc^2$, to correlated $E= h .v$, and to begin Shroedinger equation, the understanding of black hole, the universe, the sense of nano-particles in quantum dots and electron tunneling.

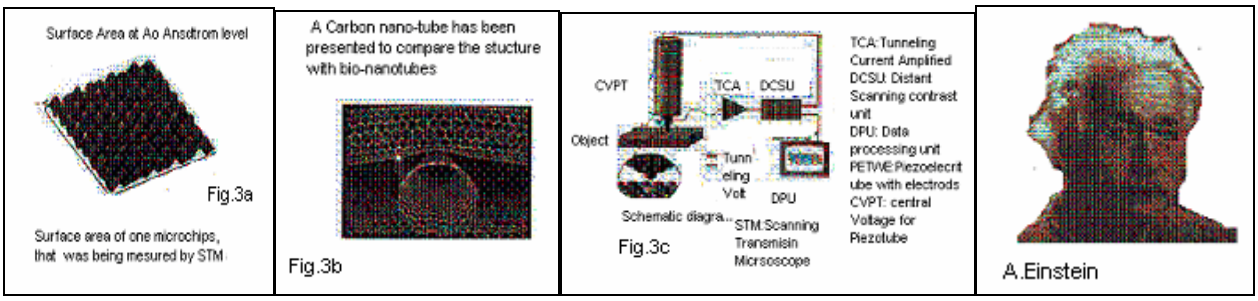


Fig.3 (a, b, c, and d) show the surface structure of 0.1 nm microchip can be modified and can be observed by STM and a how a carbon could be designed and which could be more stable than a normal carbon due to their orderly carbon structures. At the end I like pay homage to our forefather Prof. A. Einstein,Fig.3(d) as the year of Physics 2005, who showed the path to the world, that energy remains constant only it conversions are different.

Literature cited.

[1] Lijima, S (1991) Helical Microtubules of graphite Carbons, " Nature" 354: 56.].

[3] Krijn and John, 2000, Carbon Nano-fibres: Catalytic synthesis and applications, Cataly. Rev. Sci and Engg, 42: 481

[2] Brahma N K.(1996). Application of Enterobacterial plasmid in Genetic Engineering and Hybridization Techniques. In Innovation technology for the Next Millennium; Tata Mc. Graw Hill, Pub. Ltd. ND; Proceedings of the Indian Engineering Congress, Inst of Engineers (India). Ban galore, Dec. 20-24.

[3] Brahma, N.K. (1999) Enterobacterial MRHA plasmid and its possible Genetic Transformation With Escherichia coli K-12 at auxotrophic Phenotypes. Indian J. Microbial. 38 (4): p: 43-50.

[4] Brahma, N.K. (1999) Genetically Engineered Hybrid 5405 E.coli K-12 fimbriae in vaccination With Balb/c mice against 026:serotype EPEC diarrhea. Ind. J. Chem. Engg. 39 (1) p: 17-

[5] Brahma N, Schumacher A, Cullum J and Saedler H. (1982). Distribution of the Escherichia coli K-12 Insertion Sequences IS1, IS2 and IS3 among other bacterial species. J. Gen. Microbiol. 128: 2229-2234.,

[6] Brinboim HC and Dolly JA(1979) Rapid alkaline extraction procedure for screening recombinant plasmid DNA, Nucleic Acid Research &: 1513-

[7]. Eckhard T (1978) Arapid method for the identification of plasmid deoxyribonucleic acid in bacteria. Plasmid 1: 584-588.

[8]Roco MC (2004) Nano-scale and Engineering: Unifying and Transforming Tools, Perspective, AIChE Journal,50: 890-897.

[10]Albany, W(2004) Understanding E47" Antimicrobial Fibre technology", www.arcoutdoors.com

[11] Fujita, H (2003) Micro machines as Tools for Nano- technology, Sringer Verlag, International Edition SIE.

[12]Das et.al. (2005) Nanotechnology, National Science Day, Nehru Museum of Science & technology, IIT-Kharagpur-721302.

Acknowledgement:

This paper has been dedicated to my Professors, Prof. S.Basu, Prof.A.K. Mitra and my wife Mrs. Tapati Brahma, who inspired me to write this paper. However the technical support of Dept of ChE/IIT-Kharagpur, is also acknowledged.