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Understanding of MRHU-plasmids, bacterial adherence and biomaterial in clinical application

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Abstract: Bacterial adherence has been recently reviewed by plasmid genetic properties of MRHU (D-mannose resistant haemagglutination of human erythrocytes) and change of surface antigens, fimbriae=pili, as an elongated protein appendages to facilitate transport of haemagglutine from cell to bacterial membrane, to lipopolysaccaride (LPS) and to hosts. The fimbriae=pili as expanding elongated protein appendages, have been recently characterised as BNT (Bionanotube) or as ionic channels. The transport of secondary metabolites, haemagglutinine passing through membrane to the external surface, may cause MRHU the colonizations. The mechanisms of MRHU (agglutinating RBC) and their pathogenic activities have been characterised against immune responses (IR) in Balb/C mice. Both the cells and fimbriae showed similar results at different time span. Based on observations, the author designed the concept of one 'Bio-material' to be used for continuous vaccinations and for slow IR, which will monitor the body infections, in case of chronic and acute bacterial diseases. The design reveals that if blood (serum) comes in contact with fimbriae=pili=BNT embeded immobilised matrix , it will cause IR, designed specifically against the infections of 08: 075: ETEC and 026:EPEC. MRHU (+) strains. These strains were essentially studied to isolate hybrid genetically engineered 5405 MRHU(+) auxotrophic E.coli K-12, non-pathogenic to Balb/c mice, produces at the same time antiadherent immune response to protect mice against fatal 026:EPEC diarrhea. The above results of HA- sero- typing and plasmid profiles supported the concept for the development of bio-materials, and matrix based slow vaccination process.

Keywords: Adherence, biomaterials, Escherichia coli K-12, infection, MRHU, plasmid.

Introduction

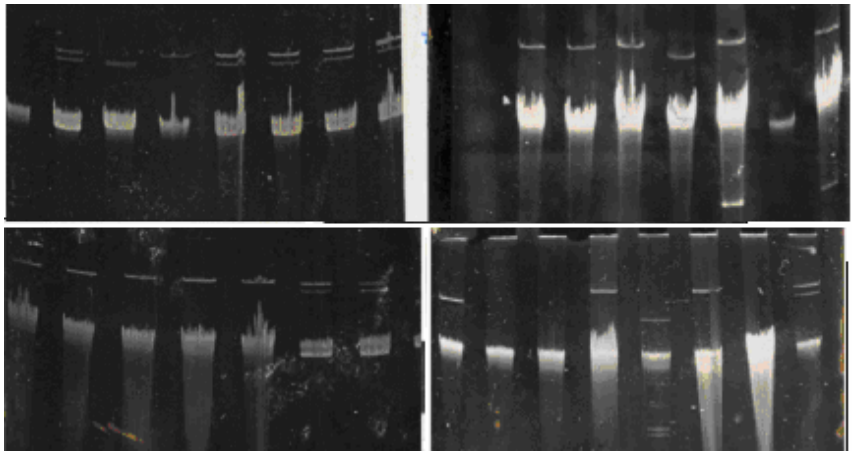
Bacterial adherence and their potentiality had been the subject of interest in the past few decades (Hohman & Wilson, 1972; Orskov, 1980; Issaction, 1977; Evans *et al.*, 1979; Beachy, 1981; Brinton, 1978; Duguid *et al.*,1955; Howick & Van Iterson, 1950). During such earlier investigations, the bacterial adherence took new shape and was studied carefully to prevent common tropical diseases like diarrhea and other bacterial contaminations. ***Escherichia coli,*** belongs to the family enterobacteriacae, was the major study source for such investigations. Of late, there has been a great spur and renewed interest in the field owing mainly to the breakthrough discoveries in the immunology and nanotechnology fields. Now, the microbial adherence gains popularity in protecting industrial products (biofouling and biocorrosion of cooling units in power plant operations) and human health care units (dental and artificial organ implantations).

To utilize bacterial adherence in medicine, the concept of antiadherent immune response (IR) signified a breakthrough in designing hybrid-blended polymer and for using the same in slow vaccination. The concept emerges from the basic idea that a hybrid fimbriae=pili can generate IR and the same could be immobilized on a matrix. The BNT (bionanotube) was conceptualized from the understanding that when a secondary metabolite of haemagglutinine protein is transported from ***E.coli*** cell to outside it first flows from inside of the bacterial cell to the cell membrane and then via fimbriae it spreads to outer surface area and then to the hosts. Several mechanisms have been considered in the molecular aspects of adherence. Among them, the concept of bacterial adherence derived by Beachy (1981) is considered appropriate in terms of adhesions or receptor interactions and the same was modified by Brahma (1993; 1999a). Bionantube (Brahma, 2006) or ionic channels have been considered to postulate the phenomena that haemagglutinine proteins are moving from cell to the hosts through porous cell membrane. The elongated protein appendages in the form of fimbriae or pili are the expansions of secreted protein, that transform in the form of BNT (bionantube) fimbriae=pili on the surface of ***E.coli,*** characterized by channel or chain like protein structure of fimbriae=pili and generates ionic clouds to attract or to adhere covalently

the surface proteins of the host tissues. ***Candida albicans,*** on the other hand, colonize or infect the outer surface of skin by damaging tissue and by secreting enzymes. Similarly dental caries may be occurred by microbes, damaging dental cements in presence of sugar (gluconic acid) for the growths.

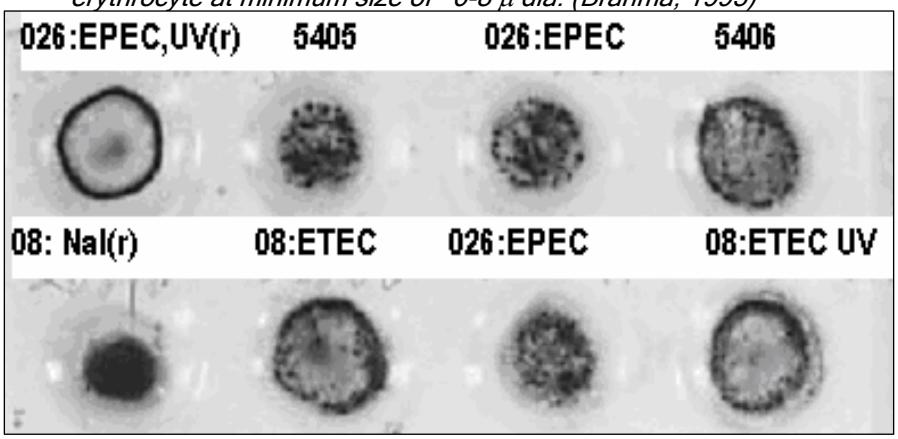
In the hybrid fimbriae vaccinations, an antiadherent IR model in mice was used (Brahma, 1999b) to design universal vaccine. In this model the cloning of different genetic properties of surface antigens, fimbriae=pili of ***E.coli*** were isolated and was cloned in a single vector plasmid pBR322, or pSU101. This served to express specific surface protein antigens or fimbriae, to support specific IR (Immune responses). The bacterial adherence was shown to be influenced by pH, temperature and genetic mutations. The mutational variations in terms of antibiotic nalidixic acid resistance were characterized by the changing pattern of MRHU to MRBO (D-mannose resistant haemagglutination of bovine erythrocytes) as shown in Fig.1 & 2. Bacterial adherence has been thoroughly studied in veterinary medicine to treat neonates (piglet, calves) against diarrhea infections.

Fig.1. Plasmid profiles of 08:ETEC strains (left) and 026:EPEC strains (right) on AGE. All the strains were collected from the infective stool specimens



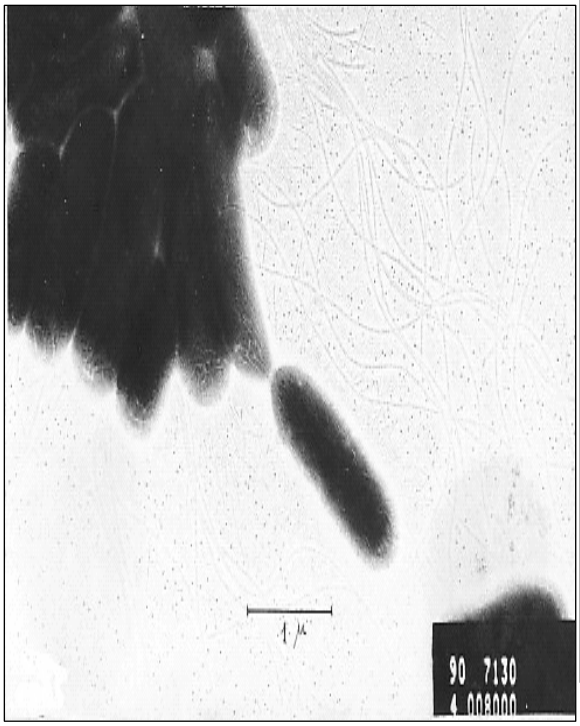
In this study, MRHU and bacterial adherence have been characterized by classical and molecular biology of plasmids genetics and sero-typing. They were used to understand the variations of different resistant plasmids, associated with the expressions of lactose and galactose in bacterial infections and formations of fimbriae as phase variations. Very recently MRHU (D-mannose resistant haemagglutination of Human erythrocytes), MRBO (D-mannose resistant haemagglutination of bovine erythrocytes) plasmids have been reviewed. MRHU plasmids has been considered as most important part of bacterial adherence along with CFA (colonization factor antigen, I, II, III.) and CS (common surface antigen, I, II) for colonization. In the present study the objective was to generate hybrid auxotrophic ***E.coli,*** strain which will be equally potential to make MRHU (+) expressions. Hybrid 5404 ***E.coli K-12*** was essential to study MRHU plasmids properties in vitro, compared to the donors. In addition to that it was interesting to know, whether the same strain can be used for anti-diarrhea vaccination, which must be nonpathogenic to

Fig.2. The variable MRHU patterns of 08:ETEC, donors and UV mutants 026:EPEC, hybrid 5405 strains. The variations of granular properties of erythrocytes developed the concepts of adherence, that they agglutinate erythrocyte cells in a chain at the ratios of 8 x 1μ E.coli per one erythrocyte at minimum size of 6-8 μ dia. (Brahma, 1993)



mice and will produce antiadhrent IR in mice. Hence, 5405 ***E.coli K-12*** hybrid was isolated and was used for some investigation on Balb/c mice. The 5405 ***E.coli K-12*** reproduced antiadherent IR in Balb/c mice against the infections of donor 026: EPEC fatal diarrhea (Brahma,1999 b). Parallel to plasmids studies in AGE (Agarose Gel-electrophoresis), regular study was also undertaken to characterize 60-65 MD MRHU-plasmid of 08:ETEC (***Enterotoxigenic E.coli)***, 026:EPEC (***Enteropathogenic E.coli***), and 70MD of hybrid 5405 ***E.coli K-12***, PLASMIDS. The characterizations of surface antigenic (SA) fimbriae=pili outermost cell wall proteins of ***E.coli*** and their isolates were essentially used in MRHU and Sero-typing. Hybrid 5405, ***E.coli K-12*** with fimbriate and nonfimbriate surface

Fig. 3. The electron micrographs of ***E.coli*** *belonging to 08:ETEC. The scale 0.1 μ provides the corresponding measure of the diameter and length of fimbriae and also the length of microbe.*

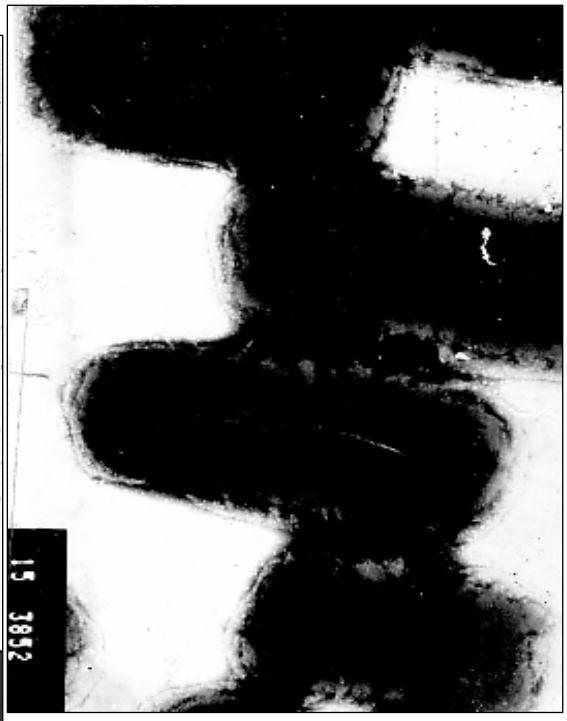


antigenic phase variations was used to study antiadherent IR in Balb/c mice (Fig. 1 & 2). Plasmids were isolated according to Brahma (1993; 1999) (Fig.1 & 2).

Materials and methods

The 026: serotype EPEC as per Brahma (1993; 1999), 08: ETEC as per Issacson (1977), Evans *et al*. 1979) and Zaidi *et al*. (1997). ***E.coli K-12 C600,*** Yale strain and hybrid 5405 ***E.coli*** strains were grown on CFA agar containing casaminic acid 1%, yeast extract 0.15%, magnesium sulfate 0.0005% and magnesium chloride 0.0005% (Brahma (1993, 1999a). Agar(2%) was added and the pH was adjusted to 7.4. As per the requirement of the bacterial growth and fimbriae, the incubator temperature was adjusted to 30 ^{o}C +/- 5^{o}C and was kept constant. In some cases 37^{o}C to 40^{o}C incubator temperatures were used when (F+ x F-) conjugations in presence of temperature sensitive ΔT^{o}C, kan ® (resistant) ***E. coli*** were carried out. Electron microscopic observations were made to characterize the fimbriae and non-fimbriae properties of ***E.coli*** of donor, mutant and hybrids, isolated as single cell or as clusters of ***E.coli K-12*** cells (Brahma, 1993;

Fig.4. The cell wall surface antigenic characteristics of 5405 E.coli K-12 hybrid.



1999a) (Fig. 3; Table 1) for matching serotyping cross- precipitations of donor and mutant strains.

Results

E.coli cell's Fimbriae =pili on SDS-PAGE (sodium dodecylsulphate polyacrylamide gel electrophoresis) was studied according to Brahma (1993). Isolation and characterizations of hybrid GE (genetically engineered) ***E.coli K-12***, 5405 MRHU (+) auxotrophic strain were

made according to Brahma (1993; 1999a). Their IR (immune response) to Balb/C mice was studied according to Brahma (1999 b), Marx (1980) and Reed *et al*. (1938). The kinetic (time dependent) MRHU formations of HA on Sera-plates and the immune responses in Balb/c were carried out according to Brahma (1993). Hybrid membrane formations and their uses were envisaged to generate inhibitions of infections by continuous IR. Balb/c mice IR were studied according to Brahma (1999 b, 2003, 2007) and Weigle and Yager (1999).

Experiments were carried out on HA-; sero-typing, fimbriate, nonfimbriate protein appendages and plasmids according to Salit and Gotschlich (1977), Webers *et al*. (1980), Evans *et.al*. (1979) and Zaidi *et al*. (1997). Fig 3, represents the electron micrographs of ***E.coli*** belonging to 08:ETEC, whereas, Fig.4 represents the surface antigenic properties of hybrid 5405 ***E.coli K-12***. Fig.5 shows filamentous protein of isolated fimbriae. Fig.6, represents SDS-PAGE of 08:ETEC, 026:EPEC, hybrid ***E.coli K-12***. Fimbriae protein concentration was measured according to Warburg and Christian (1941). The concept of micro fluidic activities of nano-channel was studied according to Brahma (2006, 2007) and

Table 1. Serotyping cross- precipitations of donor and mutant strains.

Strains and Serotyping	20075a 026:	20075aUV NT	5405 NT	5406 NT	20075a NTG, NT	20800 08:	20890 UV, NTG conjugate of *E.coli* K-12 and nal® 08:
AS: 026:	+++	+++	---	+++	+++	---	---
AS: 026 # 026: UV NTG	+++	+++	---	---	---	---	---
AS: 08: (#) AS K88	+++	---	---	---	---	---	---
AS: 08:	---	---	---	+++	---	+++	+++
AS: 08: (#) 08: nal® mutant	---	---	---	---	---	+++	---
AS: 0: K17	---	---	---	---	---	---	---
AS: 018: (#) 018: K(-): pil(-)	---	---	++	++	---	++	---
AS: K-12	---	---	++	++	---	---	+++
AS CFA-I	---	---	---	++	---	+++	----
AS CFA-II	---	---	+++	++	---	+++	----
MRHU	+++	+	+++	+	---	+++	----
MRBO	+	---	---	---	---	+++	+++
MRCH	NT	NT	NT	NT	NT	NT	NT
Fimbriae=BNT=pili	---	----	+/-	+++	---	+++	+++
E.coli lac(-)	+++	+++	+++	---	+++	+++	+++
E.coli gal(-)	+++	+++	+++	+++	+++	+++	+++
IS1	NT	NT	+++	++	NT	++	++
Lambda(λ)RT	---	---	++	+++	---	---	---

Fig.5. Filamentous protein figures of the isolated fimbriae

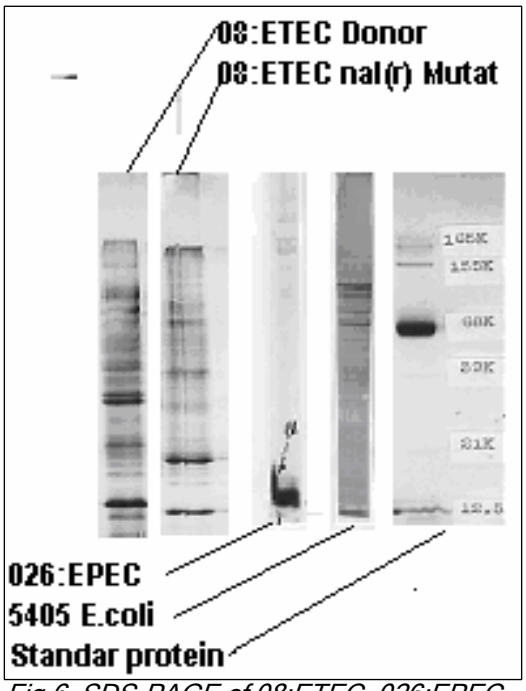


*Fig.6. SDS-PAGE of 08:ETEC, 026:EPEC, hybrid **E.coli K-12.***

Weigle *et al* (1999). ***E.coli K-12*** C600, Yale strain 5405 auxotrophic, MRHU (+) hybrid strain were isolated and their IR (Immune response) against 5405, were studied according to Brahma (1999 b). The 5405 ***E.coli K-12***, MRHU (+) autotrophy revealed two important microbial criteria: (1) MRHU (+) factor should be identified for bacterial adherence and not for the cause of pathogenicity of microbe. (2) The strain will support antiadhrent IR to Balb/c mice and the concept of vaccination, developed specifically against fatal diarrhea of 026:Sero-types EPEC. Balb/c mice. LD_{50} was estimated and studied against donor, mutant and hybrids according to Marx (1980), and Reed and Muench (1938).

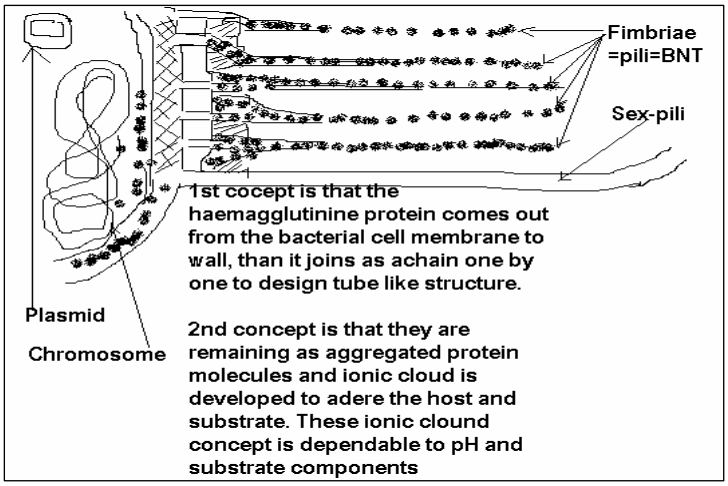


Fig.7. Possible MRHU transport through bacterial cells. It explains, how the movements of haemagglutinine proteins, as interacted molecular chains in the form of micro fluids can be conceptualized.

Discussion

Based on the experimental observations stated above, the author designed the concept of slow IR in Balb/c mice and a concept of slow vaccinations and or drug delivery. To validate the concept of one such "Biomaterial", that can work continuously for activating IR, genetically engineered hybrid 5405 ***E.coli K-12*** auxotrophic fimbriae=pili or BNT were extracted and were blended with araldite polymer. Water flow, negative to pure araldite and positive to pili=fimbriae=BNT blended hybrid polymer, satisfied the concept that a bio-resistable polymer could be useful to blend hybrid fimbriae=pili=BNT and could be implanted in the body of Balb/C to study Host-Vs-Graft immune response (IR). The concept provides furthermore that if blood fluid (serum) flows through BNT or touches to BNT, BNT will trigger IR of specific diseases in a continuous and in immobilized modes, which are cost effective and could be "universal". However a genetically engineered ***E.coli K-12*** (Brahma, 1998; 1999b; 2003a, 2003b, 2003 c; 2006) would be essential to design the matrix based vaccine delivery and to extract fimbriae with different IR specific molecules. A thorough knowledge is needed on the molecular genetics of the protein molecule. Different ETEC (***Enterotoxigenic Escherichia coli***), EPEC (***Enteropathogenic Escherichia coli*** and UTI (***Urinary tract Escherichia coli***) strains at 06:, 075: ETEC and 026:EPEC (Hohman & Wilson, 1972; Beachy, 1981; Smith & Helfer, 1973; Orskov, 1980; Issacson, 1977; Webers *et al*. 1980; Evans *et al*.,1979; Duguid *et al*.,1955) were isolated and collected to study the presence of MRHU (+) plasmids and to isolate specific haemagglutinine genes, as used to clone HA-gene into vector plasmid and to get its specific immune responses (IR), administered against specific microbes. It has been observed that the variations of bacterial adherences were effected according to the change of their serotypes. So it can be concluded that in a single 3 to 5 kilo base pair or 4 to 5 MD plasmids ring, two to three multiple genetic information can be tagged with the known map of pBR322 and / or other vector plasmids. Immune responses as caused by different protein molecules would be responsible to provide and to activate IR to mammals. The interacting proteins from different microbial diseases can be cloned in *E.coli*. This approach could be a new challenge for future vaccinations and can be used against various diarrheal infections. A new hybrid

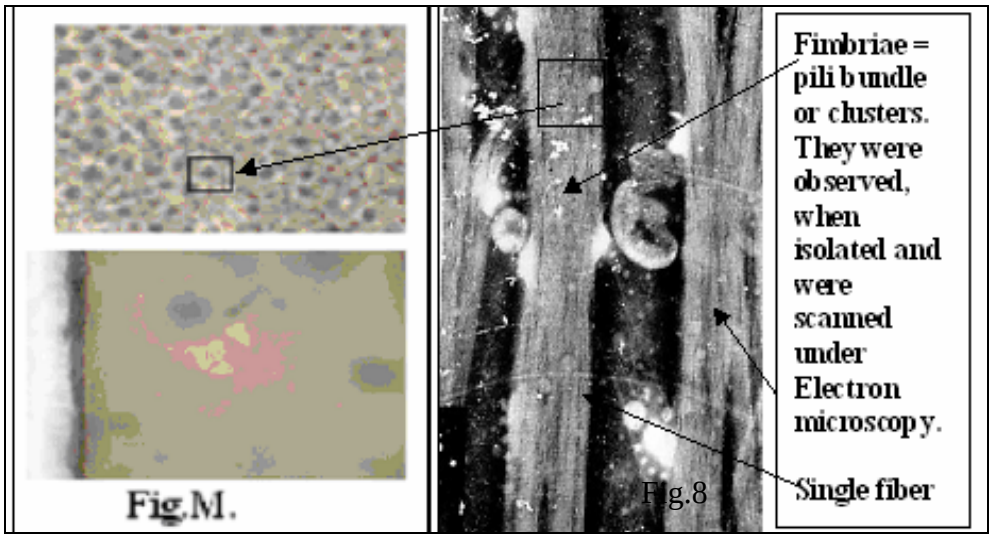
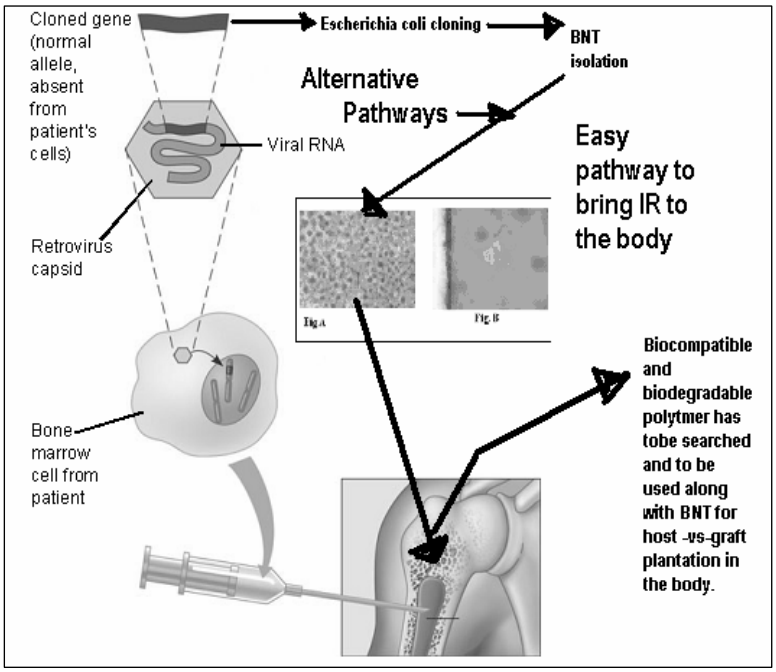


Fig. 8; Fig.8M. Change of araldite polymer surface in presence of fimbriae

biomaterial of araldite has reproduced the concepts of nanochanneling properties of fimbriae=pili, bionanotubes. The transport of haemagglutinine protein from cell to the hosts may be considered in following molecular chain movements (Fig. 7) (Brahma, 2004; Kolmalz *et al.*,1999) to make possible MRHU. It explains how the movements of haemagglutinine proteins can occur. As interacted molecular chains, the form of micro fluids may develop fimbriae =pili that will carry HA protein out of the cells and will form elongated protein appendages. However the real movements of haemagglutinine protein from cell to the hosts or to substrates are need to be studied with the help of fluorescence marker, hypothetically tagged with MRHU plasmids gene. Fluorescence gene will express along with MRHU and could be visualized under electron microscope, while HA-protein will pass from cells to the hosts. Fig.8 shows the difference of frimbriae=pili=BNT embedded (blended) araldite matrix compared to pure araldite at x400 times magnifications (Hohman & Wilson,1972; Beachy,1981; Braude *et al.*,1978; Brahma, 2003; 2007). Fig 8 M represents the change of araldite polymer surface in presence of fimbriae. Fig. 8 (c) represents the schematic proposed view of hybrid matrix applied in human and animal system. The method is considered to be an easy and cost effective mechanism compared to cloning of foreign gene as it is introduced into blood cells of bone marrow. The cloning of foreign gene is based on tissue culture involving viral propagation and to inject the same hybrid cells

Fig. 8 (c). The schematic proposed view of hybrid matrix applied in human and animal system as an easy and cost effective mechanisms for continuous vaccinations against diseases compared to cloning of foreign gene into blood cells.



into blood streams of bone marrows to repair, and to monitor the infections (Brahma, 2003; 2004; 2007 and Kamholz *et.al*., 1999).

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