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Emergence of Permeability Property in Self-sustaining Autoreplicative Protocell- like Microstructures *Jeewanu*

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Abstract

The self-assembly and self-organisation of amphiphilic molecules led the formation of limiting boundary structure around protocell-like entities. The primitive selective permeability property of membrane compartment increased the complexity of metabolic network and internal structure. If these metabolites are not quickly used in subsequent reactions they would leak into the environment. The 1:2:1:1 *Jeewanu* mixture (Bahadur and Ranganayaki 1970) shows the photochemical formation of self-sustaining, autoreplicative protocell-like microstructures "*Jeewanu*". *Jeewanu* have distinct structural organization and are able to show some properties of biological orders viz. growth from within and multiplication by budding. They have distinct double walled limiting boundary. The histochemical characterization shows the presence of some compounds of biological interest and enzyme-like activities. The present investigations shows that only water molecules cross the limiting boundary structure of *Jeewanu* except other organic solvents. These findings shows the emergence of primitive selective property in limiting boundary structure of *Jeewanu*.

The permeability property plays a pivotal role in sustaining, auto replicative and quasi-metabolic behavior of protocell-like structure. The findings contribute to our understanding of how early prebiotic structures may have acquired essential cellular properties and offer new perspectives in the study of artificial life and synthetic biology.

These findings shows the emergence of primitive selective property in limiting boundary structure and also support the "Metabolic First" theory.

Keywords: Auto-replicative, *Jeewanu*, Primitive selective permeability property, Protocell, Self-assembly, Self-organization, Self-sustaining. Permeability, protocell, prebiotic synthesis, origin of life, self-sustaining systems.

1. Introduction

Scientific studies suggested that in the environment of the primitive Earth, a series of chemical reactions produced the ingredient of life. On these substances the amphiphilic molecules might be the first player in the evolution from molecular assembly to cellular life [1-2] In appropriate concentration the amphiphilic molecules starts to assemble due to hydrophobic interactions [3-4].

The self-assembly, self-organization and interactions between compounds plays a significant role in the shape formation, aggregation and folding of molecules as well as molecular recognition. These processes led to the formation of an organized structural configuration with emergent properties of biological orders; The Protocell.

It is widely accepted that life began with simple, self-sustaining, membrane-bounded microstructures — called protocells. Protocells are composed of molecules that are not themselves alive and emergent properties of evolution [5-6]. The Protocell have a metabolic network, information components, self-replicating genome [7] and a membrane compartment that can growth and divide [8-9].

The compartmentation and boundary structure is essential for primitive life process such as Protection of biomolecules from degradation, Formation of chemical gradients, mediate a number of essential functions, capture of energy and its transduction and arrangement of metabolic network [10]. Membrane can accelerate the polymerization of RNA mononucleotides and amino acids [11-12]. It is assumed that a similar boundary structure would be required for the origin of cellular life [1]. Permeability property and the role of the enclosed molecule in the process of origin of life was discussed [10]. The primitive membranes are composed of a collection of self-assembled molecular elements that by themselves are non-living [6]. The main constituents of plausible primitive membranes are amphiphilic fatty acid and their derivatives [8]. The amphiphilic molecules play a significant role in the emergence of permeability property of membrane. The permeability of membrane provides a simple mechanism for the uptake of useful solutes into the Protocell from the surrounding.

The selective permeability of simple building blocks through membrane has been studied in a variety of vesicle compositions viz. amino acids [13], water, CO2 and ions [14], nucleotides [15], small electrically neutral solutes [16] glycerol and formaldehyde. Low level of permeability were found for many large ions [17] and organic molecules [18].

The selective permeability of membrane plays a significant role in the maintenance of definite structural organization, for acquiring nutrient substances from primitive metabolic process, growth and encapsulate the synthesized materials and also for the release of inhibitory side products. The solute flux across membrane can be increased vesicle size ie. Membrane surface area. The selective permeability property of molecular building blocks is the dynamic nature of lipid membrane. This process allows for growth and division [19], and replication [20]. The permeability of membrane provides a simple mechanism for the uptake of useful solutes into protocell from the surrounding.

Current studies suggested that the selective permeability of membrane is effected by environmental factors (temperature and pressure of solution), shape and size of molecules, solute hydrophobicity, and addition of solutes including salts, buffer, biomolecules and other chemical solvents. Permeability decreases with increasing molecular size. The electrostatic interactions of bilayer causing membrane disruption [11].

In the plausible prebiotic environmental condition, the emergence of permeability property in primitive biomembrane felt significant to investigate the emergence of permeability property in photochemically formed self-sustaining protocell-like entities "Jeewanu". Bahadur et. al. [21-25] reported photochemical formation of auto-replicative, self-sustaining, protocell-like microstructures "Jeewanu" In sterilized aqueous mixture of some inorganic and organic substances. The term 'Jeewanu' is a sanskrit word "Jeewa" life and a 'Nu' the smallest part of something. Jeewanu have a distinct structural configuration. They were able to show some properties of biological order. They grow from within, multiply by budding and showing metabolic activities. They were able to show the presence of some compounds of biological interest viz. amino acids, that are present in free as well as in peptide combination [21] nucleic acid bases as purines as well as pyrimidens [22;26], sugars as well as ribose as well as deoxy ribose [21] and phospholipids -like materials [27] in them. Jeewanu mixture is able to show the presence of various enzyme-like activities viz phosphatase, ATPase, ester–ase, and nitrogenase [21, 22, 28], Thiamine pyrophosphatase-like activities [15].

The histochemical observations show the histochemical localization of some compounds of biological interest viz Phospholipid like, basic cytoplasm-like, acidic material-like, Calcium ion-like and material [18, 30]. The histochemical observations also showed the RNA like-material and primitivity of RNA like materials [32], Jeewanu are photochemically synthesized in laboratory simulated minimal optimal environmental conditions [33].

Jeewanu fulfill the minimal life criteria suggested by Ganti [34] and satisfies maximum criteria suggested by Pohorille and Deamer [35]. Cairns Smith [36] argued that the earliest energy transferring system was photoautotropic in nature and was possibly having similar to Jeewanu.

So an attempt was made to understand the permeability property in photochemically formed protocell-like microstructure 'Jeewanu'.

2. Methodology

Method of preparation of Jeewanu (Bahadur and Ranganayaki, [22]

The following three solutions were prepared -
4 % Ammonium molybdate (w/v)
3 % Di-ammonium hydrogen phosphate (w/v)
Mineral solution - It was prepared by dissolving following 20 mg. each of Potassium dihydrogen ortho phosphate, Sodium chloride, Magnesium sulphate, Potassium sulphate, Calcium acetate, Manganous sulphate and 50 mg. of Ferrous sulphate. The above salts were dissolved in 100 ml. of distilled water.

The solutions no. 1, 2 and 3 were taken in separate, separate conical flasks and cotton plugged; the above solutions were sterilized in an autoclave at 15 lb. pressure for 30 minutes.

After cooling, 4% ammonium molybdate solution (1 volume), 3% Di-ammonium hydrogen phosphate (2 volumes) and mineral solution (1 volume) were mixed in a sterilised conical flask. 36% Formaldehyde (1 volume) was aseptically added in the above solution.

These 1:2:1:1 mixture is known as 'Jeewanu Mixture'. These Jeewanu mixture was equally distributed into eight conical flasks numbered 1 to 08. All the flasks are plugged with cotton plug. The flask no. 1 is covered with several folds of black paper known as control. Flask no. 1 to eight are similarly exposed in sunlight for variyimg periods of exposure (viz. 30 minutes, 1,4,8,16,24 and 32 hours) for photochemical formation of Protocell-like microstructure 'Jeewanu'.

Preparation of air dried samples

The particles of different periods of exposure were separated by decantation and air dried in a vacuum desiccator. The precipitates of different period of exposed were further divided into 5 sub sets (no. 1 to 5).

Suspension of Jeewanu in different solvents

0.50 mg. of precipitates of all samples (no.1 to 5) were separately immersed in 5.0 ml. of various solvents viz. ethyl alcohol, acetone, ether, deuterium trioxide, and distilled water respectively.

3. Results and Discussion

The optical and electronic prob microscopic observations shows that photochemically formed protocell-like microstructures are spherical in shape, blueish in colour, have a double walled boundary and intricate internal structure. They were capable of showing growth, multiplication by budding and histochemical localization of Phospholipid-like material on outer limiting boundary. (Fig. 1 – 6).

The morphological and internal organization of Jeewanu immersed in organic solvents (set no. 1 to 4) remained intact up to 340 hours. Jeewanu suspended in distilled water (set no. 5) showed disorganization in their structural organization. The details of microscopic examination were summarized in table no. 1.

Microscopic examination of suspension of Jeewanu at different exposure in distilled water revealed that the Jeewanu of lower exposure were quickly decolorised, ruptured and showed less aggregation in time.

Finally they were transformed into faint blue coloured, thin crystalline rod like structures. (Fig. no. 7, 8, 9).

The Jeewanu of higher period of exposure were relatively dark blueish in colour, relatively more stable and showed greater affinity of aggregation. The particles of suspension are finally transformed into thick, elongated, dark blue coloured crystalline rods. (Fig. no. 10, 11, 12).

During the different stages of rupturing of Jeewanu, presence of dense blueish material was observed.

Table no. 1. Showing changes in structural organization of Jeewanu at different exposure suspended in distilled water

S. No.	Duration of exposure of Jeewanu (in hours)	Periods of stability of Jeewanu in time (in hours)		Changes in structural organization of Jeewanu in time (in hours)		
		Size of Jeewanu (0.5 – 1.5µ)	Size of Jeewanu (1.5 – 3.0µ)	Periods of stability of boundary wall	Dis-organization of central structure	Appearance of rods or crystalline structures
1	0.30	12	24	24	24	24
2	1.0	48	72	72	72	72
3	4.0	72	96	96	96	96
4	8.0	96	120	120	120	120
5	16.0	120	168	168	168	168
6	24.0	168	192	192	192	192
7	32.0	192	240	240	240	240
8	36.0	193	241	241	241	241
9	40.0	193	241	241	241	241
10	Jeewanu in experimental medium	Intact	Intact	Intact	Intact	Absent

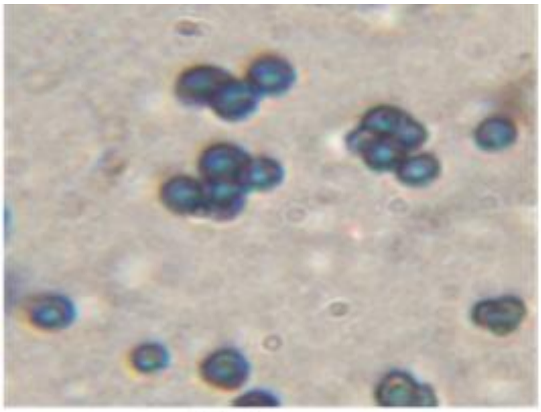

Fig 1

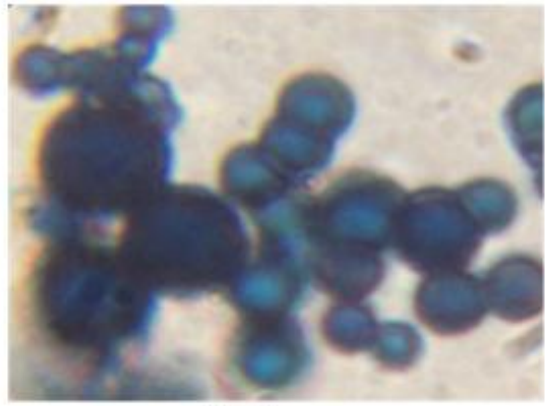

Fig 2

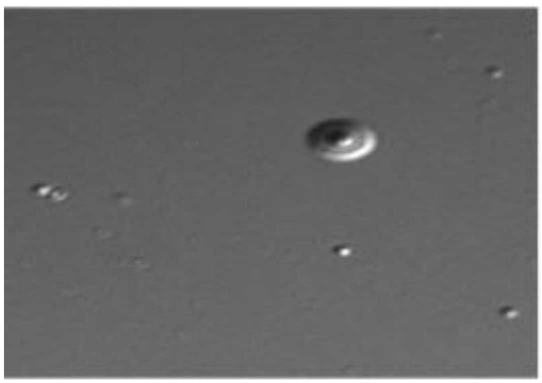

Fig 3

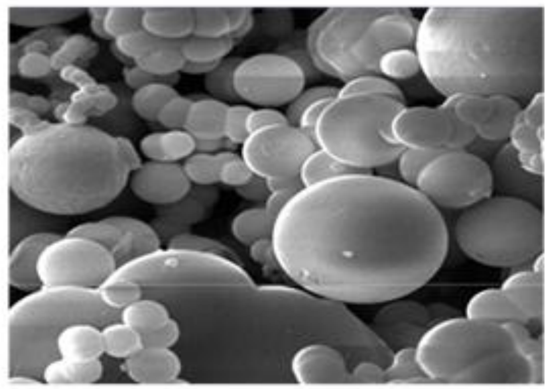

Fig 4

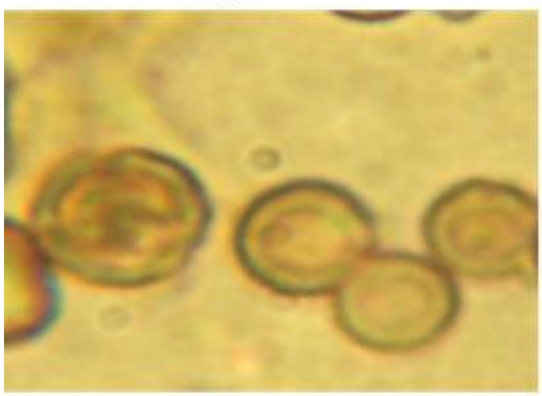

Fig 5

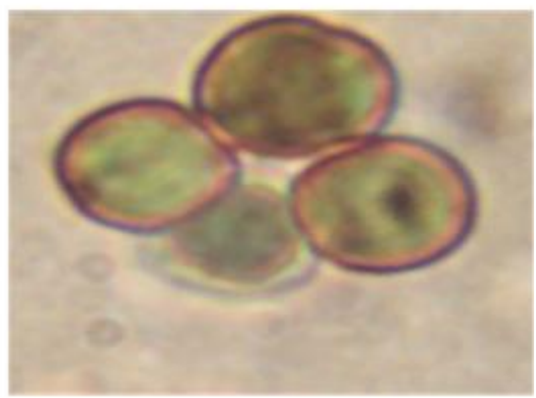

Fig 6

Fig Showing –

1. Optical micrograph of Jeewanu (1 hour exposure) Showing distinct structural organization (1500x).
2. Optical micrograph of Jeewanu (24 hours exposure) Showing distinct structural organization and budding on outer surface of the Jeewanu (1500x).
3. Confocal micrograph of Jeewanu (24 hours exposure) Showing their anisotropic morphology.
4. Scanning electron micrograph of Jeewanu (24 hours exposure) showing their surface morphology.
5. Boundary wall of Jeewanu (24 hours exposure) stained with sudan balck B (Alcoholic) (1500x).
6. Boundary wall of Jeewanu (24 hours exposure) stained with sudan balck B, prepaired in propylene glycol (1500x).

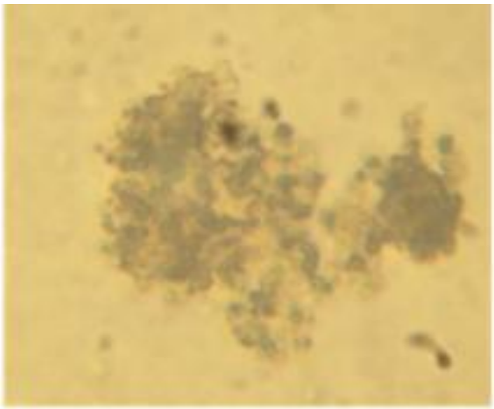

Fig 7

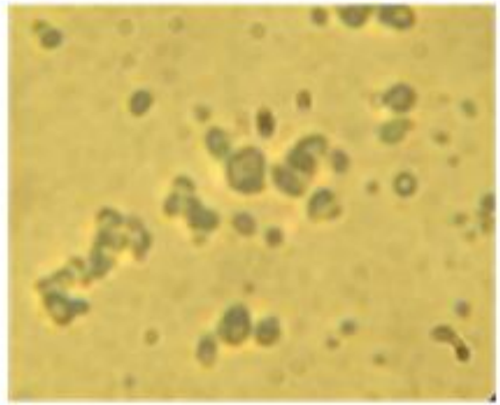

Fig 8

Fig 9

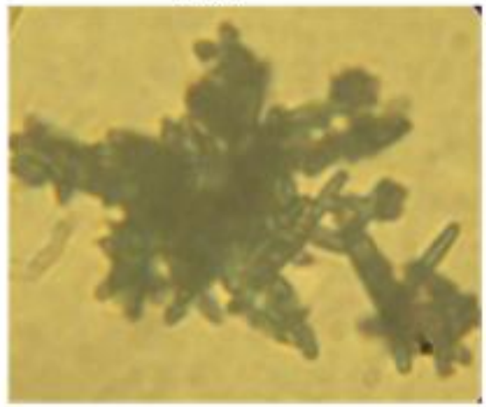

Fig 10

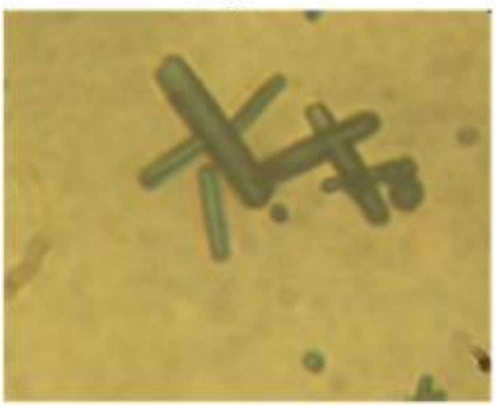

Fig 11

Fig 12

Fig Showing – Micrographs of Jeewanu showing effect on stability of Jeewanu of different exposure with distil water (1500 X)

7. Jeewanu of 30 minutes exposure, Suspended for 2 hours in Distil water.
8. Jeewanu of 1 hour exposure, Suspended for 72 hours in Distil water.
9. Jeewanu of 4 hours exposure, Suspended for 96 hours in Distil water.
10. Jeewanu of 8 hours exposure, Suspended for 120 hours in Distil water.
11. Jeewanu of 16 hour exposure, Suspended for 168 hours in Distil water.
12. Jeewanu of 32 hour exposure, Suspended for 240 hours in Distil water.

Discussion

The morphology of Jeewanu remained intact on immersion in organic solvent but was influenced by distilled water. The spherical shape of Jeewanu was ruptured and their colour was decolorized. The disorganization within the microstructure transformed the Jeewanu into crystalline rod-like structures.

The observations revealed that due to selective permeability the water molecules and other nutrients were passed inside the globule. A series of reactions were started and modified products were trapped inside the globule.

The microstructures becomes gradually turgid and these leading to structural disorganization and rupturing of Jeewanu. Finally the blue coloured spherical shaped Jeewanu were transformed into crystalline rods.

The permeation of water molecules through constituent amphiphilic molecules causes the structural disorganization from within.

Conclusion

Life originated in an appropriate site. An appropriate site would require liquid water, a source of organic compounds and a source of energy [37]. The first form of cellular life required self-assembled membrane that are produced from amphiphilic compounds on primitive Earth [38]. Fatty acid molecules are self-assembled and self-organized to form a closed compartmentalized structure [39].

Due to selective permeability property a number of polymeric products of primitive biosynthesis have been accumulated inside the vesicle. These accumulations simultaneously led the metabolic complexity [40]. If these metabolites were not quickly used in subsequent reactions they would leak back into the environment.

The 'metabolic first' theory suggested that first form of metabolism arose inside closed compartment and became more complex latter [41].

The 1:2:1:1 Jeewanu mixture shows the photochemical formation of protocell-like microstructures Jeewanu. They have distinct structural organization. They have distinct double walled boundary and intricate internal structure. The limiting boundary of Jeewanu shows the primitive selective permeability property. The permeation of water molecules causes the structural disorganization from within.

Conflicts of interest: The authors stated that no conflicts of interest.

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