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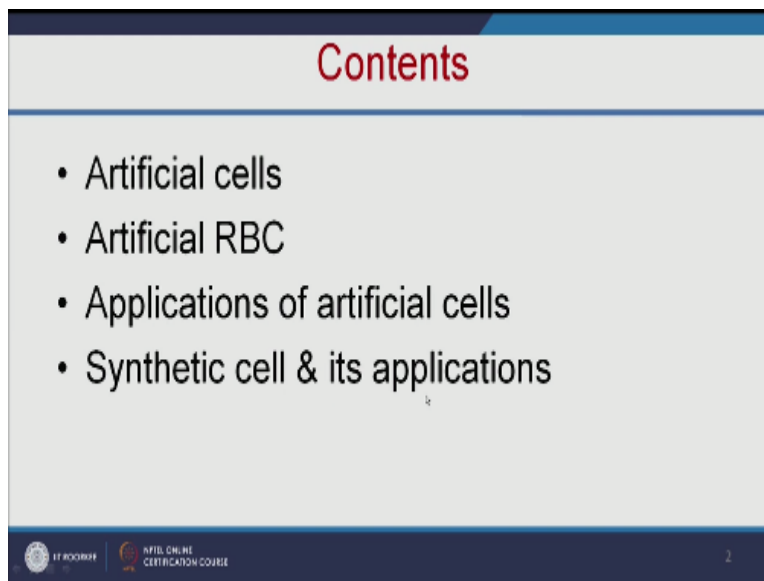
Biomedical Nanotechnology

**Lec - 14
Nano Artificial Cells**

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Hello everyone I welcome all to the 14th lecture of this course, the 14th lecture is on nano artificial cells.

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So in this lecture we are going to learn what is artificial cells, and how to make artificial RBC and also various applications of artificial cells. So in this lecture we also going to learn what is synthetic cell and its various applications.

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Introduction

- Engineered artificial cells can be used to replace dysfunctional cells in the human body and may be used in the future to treat anemia, renal failure, bone defects, and many other health problems.
- The biggest advantage in using nano materials in the fabrication of artificial cells is their small size, ranging from 1 to 100 nm in diameter (Liang et al 2008).

So in the previous lecture we have learnt how to replace the damaged tissue using tissue engineering approach. So in this lecture we are going to learn how to make artificial cell and how to replace the damaged cells using the artificial cell approach. So here this engineered artificial cells can be used to replace the dysfunctional cells and it may be used in the future to treat anemia, renal failure, and many other health problems okay.

So here the biggest advantage in using nanomaterials in the fabrication of artificial cells is their small size.

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Introduction

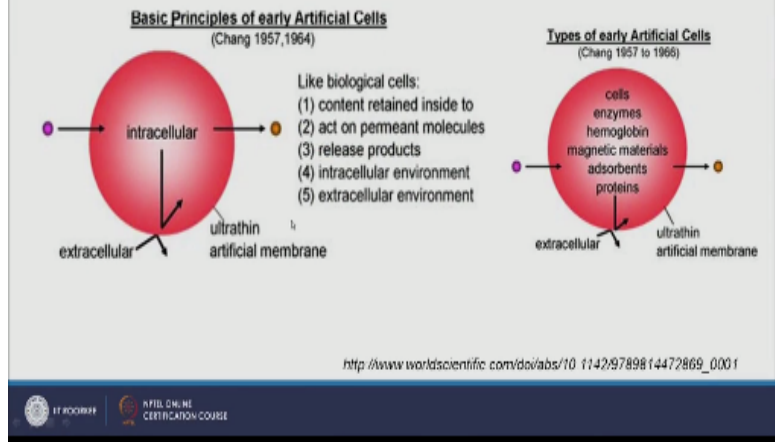
- Biological cells have a very high functional density and contain DNA coding for thousands of different proteins to carry out certain functions.
- Engineering artificial cells with the same magnitude of functional density as biological cells is a significant challenge and has only recently become feasible with advances in nanotechnology.
- The **small size allows a larger and more beneficial surface area to volume ratio** and contributes to their unique physical and chemical properties.

And as you know this biological cells have very high functional density and contained DNA coding for thousands of different proteins to carry out certain functions. So engineering artificial cells with similar function is the significant challenge okay. So with the help of nano technology we could be able to achieve those challenges. And here the small size allows a larger and more beneficial surface area to volume ratio.

So that is why when you use that nanomaterials for constraining artificial cells that could be more beneficial. So let us see the basic principles of early artificial cells.

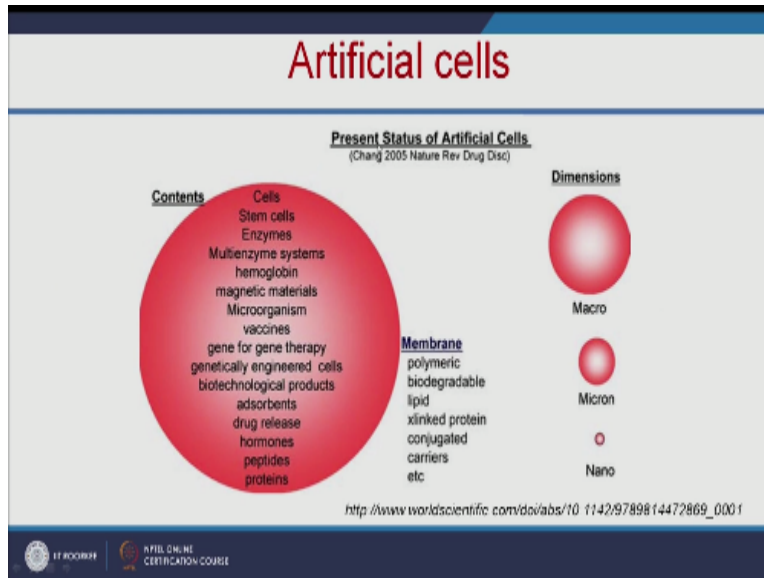
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Artificial cells



So these artificial cells are made up of ultra thin artificial membrane so it is having intracellular as well as extracellular environment. And here the content whatever you want encapsulate that will be inside the artificial membrane and this membrane is permeant okay, so it will permit the sub state and it can also release the product okay. So these are the types of early artificial cells we can encapsulate cells, enzymes, hemoglobin okay and various other materials.

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So let us see the present status of artificial cells. So you can see here we can encapsulate cells, stem cells, enzymes and everything inside the membrane, artificial membrane. So this membrane could be made up of polymeric or it could be made up of biodegradable membrane, lipid and protein based membranes okay. So these artificial cells are available in various dimensions, it can be macro or micro or nano, it depends on the cellular content which you want to encap it into artificial cells.

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Dimensions of polymeric artificial cells

- Macro dimensions
 - For genetically engineered cells, stem cells, other cells, tissues, microorganisms, etc.
- Micron dimensions
 - For enzymes, genetically engineered microorganisms and other microorganisms, peptides, etc.
- Nano dimensions
 - For blood substitutes, enzymes, peptides, magnetic materials, drugs, etc.

So let us see this in macro dimensions we can encapsulate genetically engineered cells, stem cells and even microorganisms. And in micron dimensions we can encapsulate enzymes or we can encapsulate some of the genetically engineered microorganisms and peptides and everything. And in the nano dimensions using the nano dimensions we can make blood substitutes or we can encapsulate enzymes and magnetic materials and drugs and other peptides okay.

So let us see these, how we can encapsulate these materials into this polymeric artificial cells and also what are the various applications of these artificial cells.

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Artificial cell technology

- An artificial cell or cells are systems that biomimic native cellular function to recapitulate either function and/or structure.
- Artificial cell technology seeks to address the need for a more efficient temporary if not permanent replacement.
- In contrast to tissue engineering, the field of artificial cells is concerned with singular cells and recapitulations of functions, instead of whole complex tissues.



So here these artificial cell or systems that biomimic native cellular function to recapitulate either function or the structure. And this artificial cell technology seeks to address the need for more efficient temporary solution okay. So when compared to the tissue engineering, where you get the whole tissue will be replaced here we are going to focus mainly on the single type of cells okay. So in contrast to tissue engineering the field of artificial cells is concerned with singular cells okay, instead of the whole complex tissues.

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Artificial cell technology

- The wide range of artificial cells can be glimpsed in the various applications that arose ranging from whole cell encapsulations of **pancreatic islet cells and hepatocytes**, liposome encapsulation of hemoglobin(Hb), and polymerized Hb.
- In the treatment of enzyme and single system defects, the application of whole cells may be detrimental, and replacing the enzyme or the single system may be more efficient as is the case with artificial red blood cells.
- The application of **nanobiomaterials is necessary to both better biomimic cellular systems and construct a more efficient system than nature itself.**

So for example, here the pancreatic islet cells can be encapsulate into polymeric capsules and it can act like your pancreatic cells to produce insulin in case of diabetic patients. And it can also encapsulate hepatocytes, so that can be useful for the patient with the liver failure okay. And in some cases like in the treatment of enzymes and the single system defects, so we do not need even the complete cell okay, we can encapsulate only the particular enzyme.

So the application of whole cells may be detrimental in case of single enzyme defects okay. So replacing the enzymes or the single system may be more efficient in those cases. And again the application of nano biomaterials is necessary to both better biomimic the cellular system and also to construct more efficient system than the nature itself. So using these artificial cell technology we can mimic the biological system and also we can make, better than the biological system okay. So let us see how we can make it.



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Cells in artificial cells

CELLS IN ARTIFICIAL CELLS (AC)
(Chang, 1964 Science; Chang, 1965; Chang et al., 1966)

- (1) Cells inside AC protected from immuno-rejection (antibody, wbc).
- (2) Oxygen & nutrients equilibrate rapidly into artificial cells.
- (3) Secretion (e.g. insulin) controllable by permeant material (e.g. glucose).
- (4) Conversion of waste metabolites and toxins (e.g. hepatocytes).

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And this is the cells in the artificial cells. So here the cells are protected in the artificial membrane okay. So here the cells inside the artificial cells protected from the immune rejection. So when you inject the cells directly inside the body, so that will be rejected by your immune system, but when you protect these cells in your polymeric capsules so that will be protected from the immune rejection okay, immune rejection and here the oxygen and nutrients can easily pass over this okay. And again for example, if you are using this pancreatic cells so depends on the glucose level it can secrete the insulin.

And again if you are encapsulating the liver cells into this it will convert the waste metabolite and toxins okay.

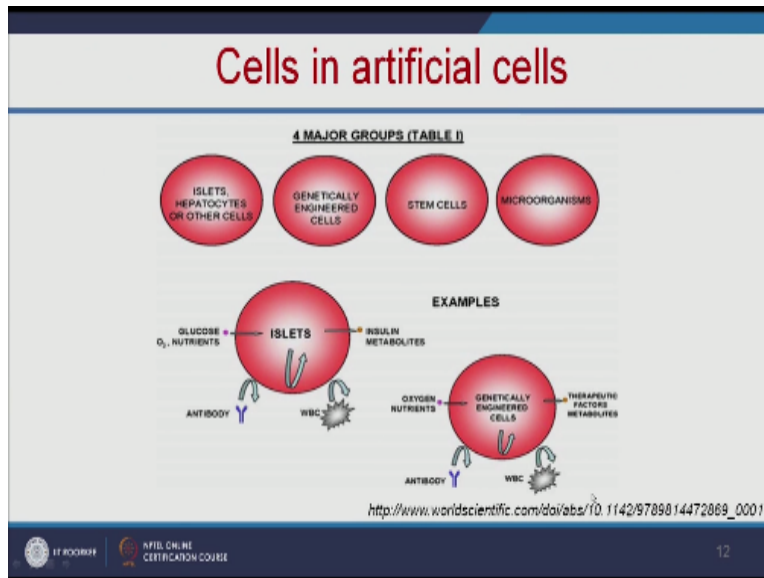
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Polymeric artificial cells containing cells

Cell Content	Aim
Cells and Tissues	
Pancreatic cells	Feedback controlled secretion of insulin for diabetes mellitus
Hepatocytes	To support liver function in liver failure
Kidney cells	To secrete erythropoietin to treat anemia
Parathyroid Cells	To secrete parathyroid hormone to treat hypoparathyroidism

So these are some of the other examples, so if you are encapsulating pancreatic cells it will be secrete the insulin for diabetes patients and if you are encapsulating the hepatocytes it will support the liver function in the liver failure. And if you are encapsulating kidney cells it will secrete erythropoietin to treat anemia. And parathyroid cells to secrete parathyroid hormone to treat hypoparathyroidism.

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So we can also encapsulate genetically engineered cells, stem cells and even microorganisms okay. So in this example if I am encapsulating these islet cells, so it depends on the glucose level it will produce the insulin and if we are using this genetically engineered cells, so it can produce various therapeutic factors that could be useful for various therapeutic applications.

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Artificial cells for cell encapsulation

STANDARD METHOD OF CELL ENCAPSULATION

cells in gel sphere membrane formed around gel sphere dissolve gel to disperse cells inside AC

(1) Cell trapped in AC membrane causing membrane weakness.
(2) Cell exposed to outside resulting in immuno-rejection.

ENLARGED

AC MEMBRANE

cell inside AC

cell outside AC

MEMBRANE

INSIDE

OUTSIDE

(1) MEMBRANE WEAKNESS

(2) IMMUNE REJECTION

- Standard drop method of preparing artificial cells to encapsulate cells results in weakening of the membrane and exposure of cells, resulting in immuno-rejection. New 2-step method solves this problem.
- This method involves first forming very small alginate gel microspheres containing cells. These small microspheres are then enclosed in larger gel microspheres.

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So let us see how to encapsulate these cells into the artificial cells, so here there are two methods the first one is standard method of cell encapsulation. So here the cells will be encapsulated in the gel sphere and followed by that the membrane are formed around the gel sphere okay, this green color is the membrane. And then dissolve the gel to disperse the cells inside the artificial cells okay.

So finally you dissolve the cells and disperse the cells into the artificial membrane. So here the major drawback is the cells trapped in the artificial cell membrane causing membrane weakness, you can see here some of this white color is your cells okay, so it is some of the cells are on the membrane. So due to which the cells expose to outside resulting in immune rejection, you can see here this white color is the cell and some of the cells are exposed to the outside environment okay. So which will lead to membrane weakness as well as immune rejection okay.

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Artificial cells for cell encapsulation

NEW 2 STEP METHOD (Chang & Wong USP 1992)

a. ALGINATE GEL SPHERE
b. IN LARGER GEL SPHERE
c. FORM MEMBRANE
d. DISSOLVE GEL INSIDE

c
d

MEMBRANE
OUTSIDE
MEMBRANE NO CELLS
INSIDE

Cells not exposed to outside
AC membrane remains intact

AC MEMBRANE
Cells inside AC

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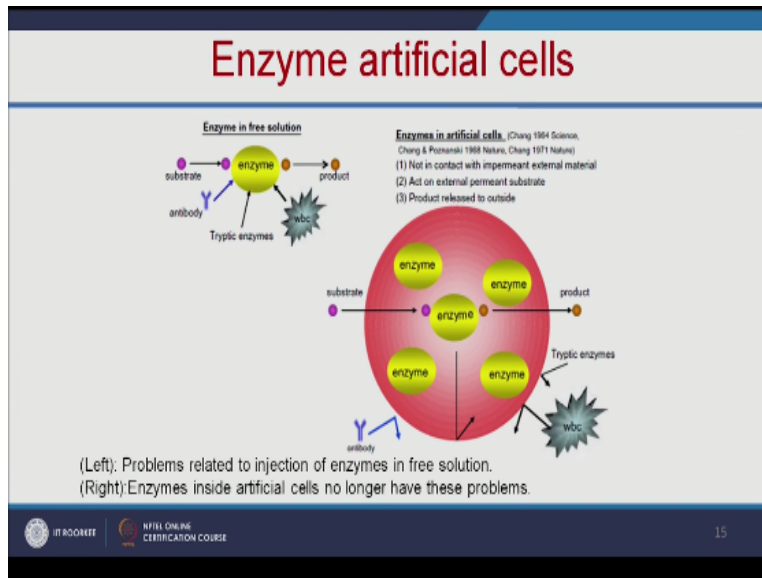
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In this way, there will be no cells on the surface of the larger gel spheres. As a result of this, no cells will be trapped in the alginate-polylysine-alginate membrane when it is formed around each larger gel microspheres.

The small alginate gel microspheres as well as the larger gel microspheres are then dissolved to release the cells, allowing them to be freely suspended in the artificial cells. Thus, the cells are dispersed freely inside the artificial cells.

So to work on this there is another method called two step method, so in the two step method the first the cells are encapsulated into the alginate gel sphere then these smaller gel spheres are encapsulated into larger gel sphere okay. So then followed by that you form the membrane, this yellow color is the membrane, so then you dissolve this smaller spheres so all the cells are evenly dissolved into the artificial cells okay. And you can see here all the cells are safe inside the membrane and there is no cells exposed to the outside. So here the cells are no exposed to outside, so this artificial membrane remains in that.

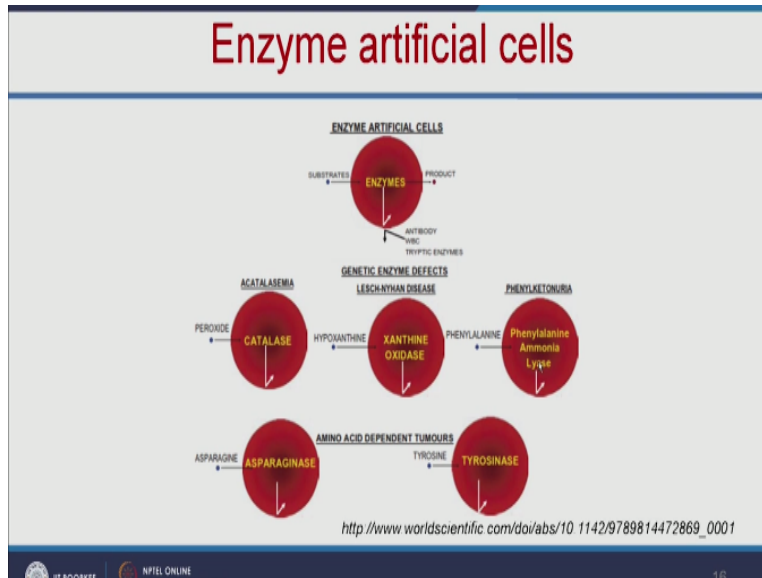
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So let us see how to encapsulate the enzyme into this artificial cells, as I told you earlier in some of the cases we do not have to encapsulate the whole cell, so if you have some enzymes defects you can encapsulate only the particular enzyme and that could be also act like a therapeutic artificial cells. So when you inject the enzyme directly what happens is, antibody will be produce enzyme or your WBC will attack okay.

And tryptic enzymes will digest to enzymes, but when you encapsulate this enzymes into artificial cells, so it is not in contact with the external material. So all the enzymes are protected in the artificial cell membrane okay, so when the substate enter inside the artificial cell and it will convert the substate in the product, and the product will be released okay.

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So let us see some of the examples okay, you can see here solid this is like acatalasemia it is due to lack of catalase enzyme and phenylketanoria it is due to lack of phenylalanine ammonia lysase okay. So when you encapsulate these enzymes into artificial cells that could be useful for treating the patients with lack of the particular enzyme. And another thing is we can also use it for cancer therapy.

For example, there are some of the tumors that are amino acid dependent tumors. For example, this enzyme like asparaginase it can remove the asparagines and also this tyrosinase enzyme that will remove the thyrocine which is recovered for melanoma growth.

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Enzyme artificial cells

Enzyme Artificial Cells implanted into body

The diagram illustrates the concept of enzyme artificial cells. It shows several green squares representing artificial cells and two red circles representing body cells. Arrows indicate the interaction between the artificial cells and the body cells. The artificial cells are shown to be implanted into the body, where they can interact with the body cells to perform specific functions.

Inborn error of metabolism (*Chang&Poznansky, 1968 Nature*)
Catalase Artificial Cells for Enzyme-gene therapy
in acatalasemic mice: congenital catalase defect

Suppress cancer growth (*Chang 1971 Nature*)
Asparaginase artificial cells suppress lymphosarcoma
in mice. Now extended by others as PEG-asparaginase for
clinical trials in leukemic patients

Suppress melanoma a fatal skin cancer
(*Yu & Chang 2004 Melanoma Research J*)

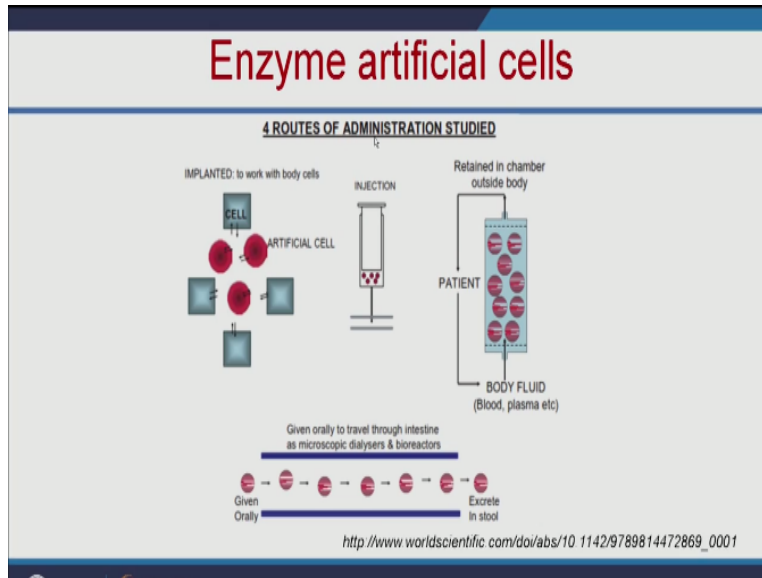
Body Cells

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So let us see some of the examples of enzyme artificial cells. For example, here this catalyst artificial cells for enzyme gene therapy was used in mice model which is lacking the catalyst enzyme okay. And we can also use this asparaginase artificial cells which could suppress the lymphosarcoma in mice model, and also it can also suppress the melanoma a fatal skin cancer. So we can encapsulate this enzyme at artificial cells and that could be useful for various therapeutic applications.

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And there are four routes of administration for this enzyme artificial cells, so it could be implanted to work with the body cells or it could be injected okay, or it could be written in the chamber outside the body. So the patient's body fluid can pass through and filter and purify and it can go back to the patient. And another thing is could be taken orally and it will be known normally by the excretion process.

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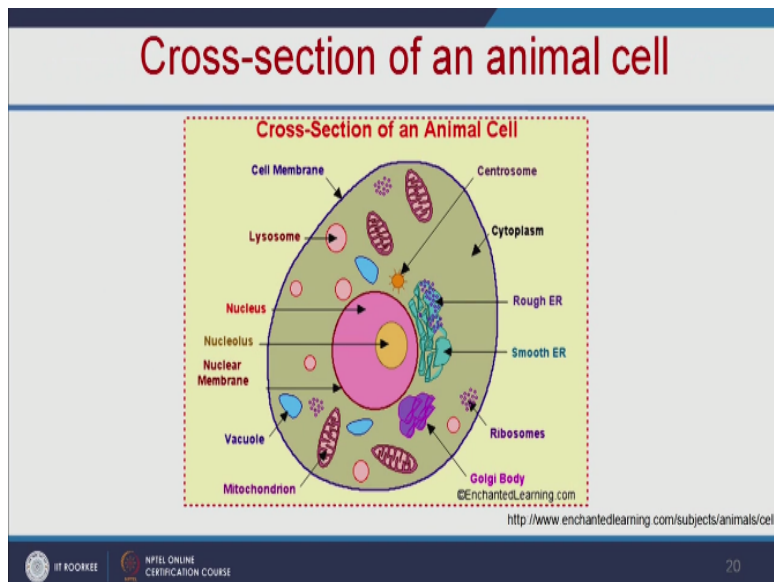
Artificial cells containing microorganisms

- Microencapsulation of cholesterol removing microorganisms-
 - *Pseudomonas pictorum*



And not only the cells or enzymes we can also encapsulate the bacteria. So you can see here the microencapsulation system cholesterol removing bacteria *pseudomonas pictorum*, so we can use this kind of bacteria to remove the cholesterol from our body.

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And these are typical cross section of an animal cell which you can see here bacteria and nucleus and various cellular organelles like mitochondria, liposome okay. So the question is can we make similar kind of artificial cells. So how to make such kind of artificial cells, you can see here this picture so each look like a small, small capsules. So the nucleus is looking like a small capsule and the mitochondria look like a small capsules.

So the idea is we can make a small, small capsules based on lipid carriers okay, and we can put those small capsules with a big size capsule. And we can make artificial biological cell okay.

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Capsosome

- A capsosome is a polymer capsule containing multiple liposomes.
- The development of capsosomes shows that the possibility of fabricating a biologically similar artificial cell or artificial organelles is not far off the horizon, and can lead to many new exciting possibilities in biotechnology.
- Another development in the realm of artificial cells is the use of silica-coated beads for *in vitro* protein synthesis.



So that is called capsomes, so the capasome is a polymer capsule containg multiple liposomes okay. So the development of capasomes shows that the possibility of fabricating a biologically similar artificial cells are artificial organelles. So we can use the liposome based small capsules, so the each capsules can act like a mitochondria or nucleus or other cellular organelles, and all those capsules will be in a big size capsules okay.

So this complete set up is called as capasome, so which could mimic like your biological cell. So the another development the artificial cell is use a silicon coat of the beats for in theinvitro protien synthesis.

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Capsosome

- Lim et al (2009) found that the beads can encapsulate transcriptional and translational machinery for the synthesis of functional proteins.
- The development of this type of nanoparticle can lead to the development of artificial cells through the parallel synthesis of varying functional proteins in designed combinations

So the limited found that we can use the beads that can encapsulated the transcript and the transmissional missionarie for the synthesis of the functional coatings. So we canuse the silica beads okay, that will have the all the ensumed transcrption and translation and it can also liked a tranrcption and translation missionary.

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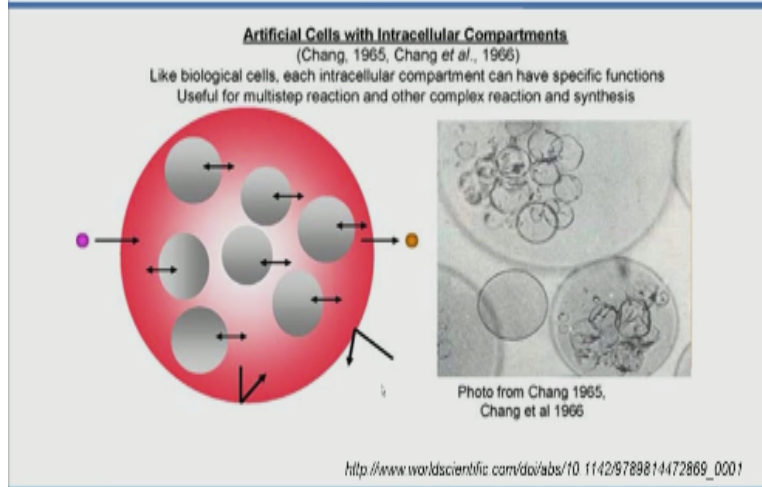
Capsosome

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And the nano particle and the second lead to the efficient artificial cell and the development okay.

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Capsosome



So let us see the capsosome so you can see here the like and the biological cell only so the each intracellular compared to the at the department have the specific function okay, so it can function like a nucleus or it can function like a mitochondria and the liposome small capsules are in the big size and the liquid based carrier and the capsosome and which mimic like a capsosome and in the biological cell.

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Magnetic materials in artificial cells

Magnetic Material & Biologics in Artificial Cells (AC)
(Chang, TASAIO 1996)

This way, external magnetic field can
 (1) direct the movement of AC
 (2) remove specific AC after reaction
 (3) retain the AC at specific site of action
 (4) Stir or agitate AC (e.g. in bioreactors)
 (5) etc.

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So the thing is the magnet material in the contribution cell in the capso zone we can also add the magnetic particles to the capso zone and the advantage is we can direct the moment of the direct cells we can move the artificial to the particular moment use the extend magnetic field and also we can remove the artificial cell after the reaction again we can retain the artifcal for the specific cell so we can retasin in the particular locationusin the magnetic field in the location and in the bioreactors using the magnetis properties we are strivct te you can additive it.

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Nano biomaterials for artificial red blood cells

- One major finding in the development of artificial cells is their possible use as oxygen carriers in the bloodstream for the treatment of anemia
- Blood substitutes, unlike blood itself, serve solely to carry oxygen and carbon dioxide throughout the body.
- A lot of the research being done focuses on hemoglobin-based products, which include PEG modified liposome-encapsulated hemoglobin, nanoparticle and polymersome encapsulated hemoglobin, and polymerized hemoglobin solutions (Sarkar 2008)
- One example of an oxygen carrier encapsulates a solution of concentrated hemoglobin and is called a hemoglobin vesicle (HbV).
- HbV was found to have oxygen-carrying capacity comparable to that of normal red blood cells (Bucci2009)

Do the blood through it will be in the nano biomaterials for the making the artificial RBC this is the red blood cells. So one of the major finding in the artificial cells is the possible use as the oxygen carrier and which could be find the treat the anemia and the other disease so here the blood circulation is substitute the main function is the carry the oxygen and the remove the carbon dioxide from the body here the lot of the research has been done some of the examples we can use the polythelin liquid in the and also in the polymer zone.

So which will act like your artificial r b c and the red blood substitute and the other examples like we use a concentrated hemoglobin and we can encrapt in the polymeric basicall. So the hemagobin have to found the oxygen carrying capacity rbc so here the hameglobin moplecules extended from thr red blood cells are modified by the encrapt in the crossing substitute and the linkage process and the stablete the hameglobin and also the allow the steleration of the products and the human in the virus and the other micro organism .

So as I told you this red blood substitutes or the made up of polymer are it will allow the r b c substitutes to sterlife which make sure that there is no viral infection of the blood sample okay. And the membrane of the artificial cells allow the permium substates and then allow as I told you earlier so the molecule enter in to the we will artificial r b c and we allow the product go out through the artificial cells.

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Red blood cell substitutes

- Hemoglobin molecules extracted from red blood cells are modified by microencapsulation or cross-linkage to produce red blood cell substitutes.
- The encapsulation and linkage processes stabilize the hemoglobin molecules and also allow sterilization of the products to remove human immunodeficiency virus (HIV) and other microorganisms.
- The membranes of artificial cells allow permeant molecules such as oxygen and substrates to enter and allow metabolic products, peptides, and other material to leave.

So the artificial are here in the artificial rbc this is product from the rejection so we are ni the cells are enter into the polymeric so the material so these are produced from the numericalogical rejectin and we need a RBC substutes in the most of the case are like the surgery in the emergency treatment the modified does not contain RBC memberane. So therefore blood group antigens, so ther vwill not need for the cross matching .

So as I told you earlier is the rbnc substutes are mainly made up of the polimers so you don't have in the blod group and the antiger on the surface so you will give this artificsial to any person like if the person is having the A group in the B group or O group there is no restriction and the artificial RBC to any person with any kind of the blood group That is the advantage of the RBC because it don't have the surface antigent okay.

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Red blood cell substitutes

- In this way the enclosed materials are **protected from immunological rejection** and from other materials in the external environment.
- There are many situations wherein modified hemoglobin has the potential to substitute for red blood cells, including surgery and in emergency treatment for severe traumatic injuries resulting from traffic accidents and other accidents that cause severe bleeding and hemorrhagic shock.
- Modified hemoglobin does not contain red blood cell membrane and therefore **no blood group antigens**, it can be used without the need for cross-matching or typing.
- Modified hemoglobin can be lyophilized and stored as a stable dried powder that can be reconstituted with the appropriate salt solution just before use.

So the another advantage is this modified hameglobin can be lifolised and stored as a stable dried powder so that just means the RBC is stored in the 43 days in case that the several month it is for the yearts the in the sallinethis is the advantage of the RBC.

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Artificial red blood cells

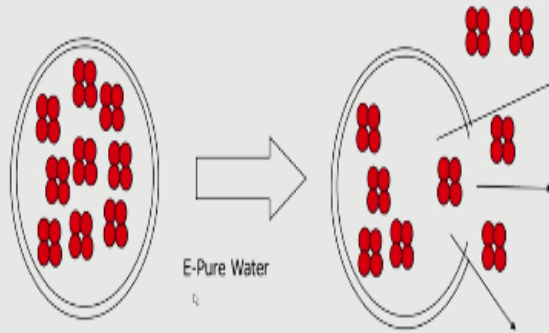
- Modified hemoglobin
 - high oxygen carrying capacity
 - do not have blood group antigens
 - longer half life
 - non toxic

So let us see some as I told artificial RBC will having in the capacity normal rbc and there is any person to the any kind of the blood group and compare to the normal RBC it will have the longer life and also it is the non toxic and okay.

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Extracting Hb from RBC's

- RBC's contain Hb which transport O_2 through body
- RBC's are lysed with E-Pure water to extract Hb

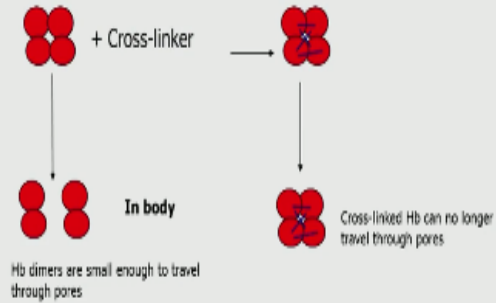


So there is a making procedure and the artificial RBC and the extract the hemoglobin in the RBC and this RBC contains the hemoglobin which is going to transfer the oxygen throughout the body so these RBC can be realized in the pure water. So just put the RBC in the water and that will lyse and it will release the hemoglobin in the inject the hemoglobin directly and there is because.

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Why cross-link Hb?

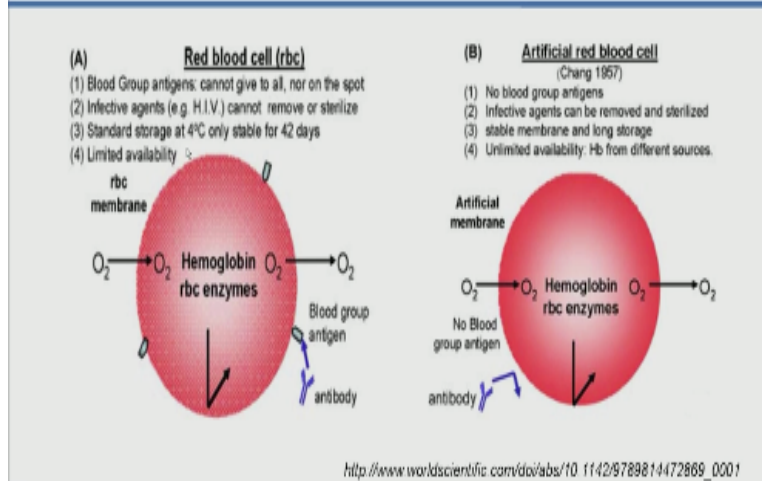
- Hemoglobin must be cross-linked when placed in the blood stream.
- Hb breaks into dimers which can travel through capillary pores (holes) and cause death.



When placed in the hemoglobin and the brakes in to the diameters and which can travel through the capillary holes and they will hemoglobin should be cross links and this cross links will be hemoglobin travel through the pose. So we can en capitulate this and the polymer in the artificial RBC. So let us see the difference between the normal RBC and artificial RBC.

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Red blood cell vs Artificial blood cells



And the RBC will have the blood group and the antigens, so we cannot give to all and we cannot give in the spot so we have to check the blood group and we have to check the compatibility and then only we will give the blood donation okay. So also the another problem is there is a chance for the infecting like H I V okay which we cannot from the blood and the another thing is we can store the RBC at 4°C for three 42 days and the some of the blood groups are in the limited variability and there is also major issue but in the case of the artificial.

So there and there is no blood group antigens' will give to any group in the sterilized in the powder form we can use it will have in the artificial membranes in it and the artificial red blood cell.

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Size of artificial red blood cells

- Several investigations have been conducted in order to find the "ideal" size requirements for nanoparticles developed as oxygen carrying artificial cells.
- Theoretically, the nanoparticles must be able to circulate freely through even the smallest of capillaries, which can be as small as 4–7 μm in diameter.
- However, nanoparticles above approximately 200 nm will be removed by the spleen through mechanical filtration and consequently accumulate in the spleen.
- On the other hand, nanoparticles with diameters below approximately 70 nm will be removed by the liver.
- As a result, it has been suggested that the 70–200 nm range is optimal for intravenous delivery and circulation.

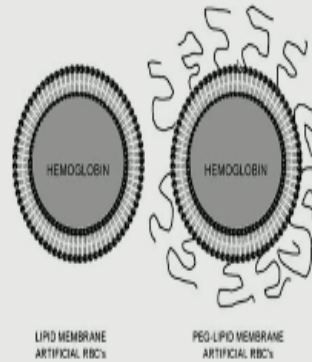
So when you make the artificial cells so the size is very important parameter so the seven investigation has been done in the ideal size for the artificial RBC. So the theoretical on the normal RBC or in the between 4 to 7 micrometer okay, whenever we make the nanoparticle more than the 200 nano meter. So that will be removed by your spleen and the 70 nano meter and that will be removed by the liver so we have to make the nano meter which has been 70 to 200, so that it will be use full for the circulation.

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PEGylation

- Artificial cells modified with PEG have longer circulation times

- Incorporation of polyethylene-glycol (PEG) to the lipid membrane resulted in marked improvement in the circulation time of hemoglobin PEG-lipid vesicles (PEG-lipid membrane artificial rbc's).



We can increase the circulation time by the adding the P G so that is called as the percolation and they will peculation. So we can add this poly thane glycol so that will improve the circulation and the it will improve the stability of the artificial cell.

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Nanodimension artificial rbc's

- Nano dimension artificial rbc's should have the following properties
 1. Contains little or no lipid in the membrane.
 2. Persist in the circulation after infusion for a sufficiently long time.
 3. Be stable in storage.
 4. Remains stable after infusion for the duration of its function as a blood substitute — but it has to be biodegraded soon after the completion of its action in the body.

The nano dimension have the following properties and in the sufficient amount it is stable in the duration as the blood substitute and after the function over they will be biodegraded in the membrane material and it is toxic.

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Nanodimension artificial rbc's

5.The membrane material and its degradation products have to be nontoxic.

6.In addition to hemoglobin, the nano artificial cells should contain important rbc enzymes like superoxide dismutase, catalase, carbonic anhydrase and methemoglobin reductase.

7.The membrane should be permeable to reducing agents and/or glucose.

The toxic and the addition to the hemoglobin in the nano artificial cells could contain for the catalyst in the carbon anhydrides and the other enzymes okay. So the artificial RBC is the function of the is not only carrying the oxygen so it has to remove the carbonados oxide and it should also remove the radical it should have some other enzymes artificial RBC so that it can exactly mimic like your ordinal RBC and the membranes should be.

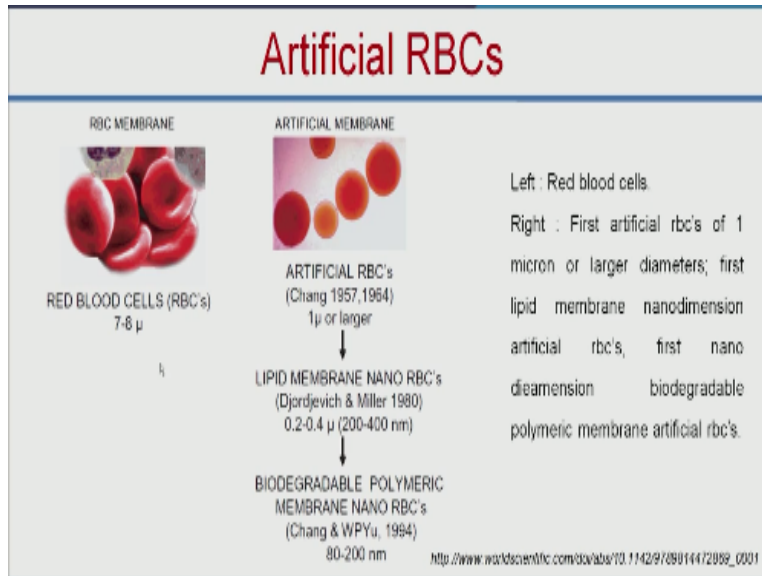
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Nanodimension artificial rbc's

- 5.The membrane material and its degradation products have to be nontoxic.
- 6.In addition to hemoglobin, the nano artificial cells should contain important rbc enzymes like superoxide dismutase, catalase, carbonic anhydrase and methemoglobin reductase.
- 7.The membrane should be permeable to reducing agents and/or glucose.

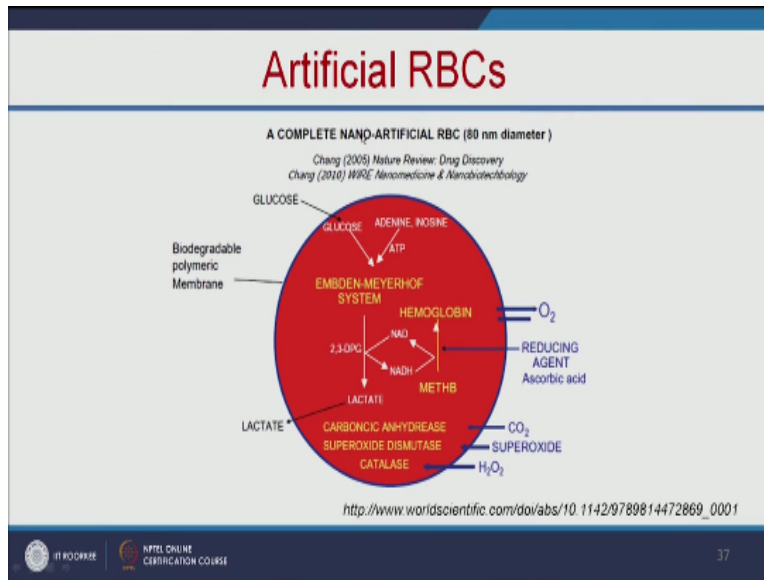
So the permeable to the reducing agents or the glucose okay, so the membranes also so be permeable. So that it can be in take the glucose and it can convert in to the product. So let us see the difference see here the normal size is between.

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And the micrometer and the artificial are in the first artificial RBC in the size of one micrometer or the larger and the later the size is reduced to the 0.2 to 0.4 micron and that is okay the 200 400 nano meter it is the lipid membrane and the nano RBC and now what ever having is bio degradable polymeric membrane and in the RBC here is the size between 80 to 200. So that it could escape from the liver as well as spleen.


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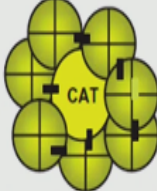
So this is the complete nano artificial RBC you can see here it is having hemoglobin for carrying oxygen and also it is having enzymes like carbonic anhydride for moving the carbon dioxide and it is also having enzymes like super oxide dismutase and catalase to remove the free radicals in your blood.

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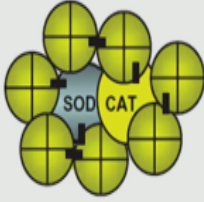
Artificial cells as oxygen carrier



Polyhemoglobin (PolyHb)
Chang, Science 64, BBC 71





PolyHb-catalase
Chang BBC 1971



SOD CAT
PolyHb-CAT-SOD
D'Agnillo & Chang,
Nature Med 1998

http://www.worldscientific.com/dovabs/10.1142/9789814472869_0001



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So let us see some of the applications of artificial cells so artificial cells as oxygen carrier so we can have a polyhemoglobin so that will have more amount of oxygen carrying capacity. And we can also add SOD that is superoxide dismutase and catalase enzymes so that will remove the free radicals.

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Applications of artificial cells

ARTERIAL OBSTRUCTION: STROKE, INFARCTION, ETC

Red blood cells

O₂ Lack (Ischemia)

PolyHb solution

polyHb-SOD-CAT solution

Arterial obstruction from the narrowing of the artery can result in stroke and heart attack.

Red blood cells, being 7 to 8 micron in diameter, have difficulty flowing through obstructed vessels to supply the needed oxygen.

PolyHb, being a solution, can perfuse through to supply the needed oxygen.

However, if oxygen lack is prolonged, reperfusion with an oxygen carrier can release damaging oxygen radicals.

One possible solution is to use PolyHb-SOD-CAT that has the dual function of being an oxygen carrier and having the ability to remove oxygen radicals.

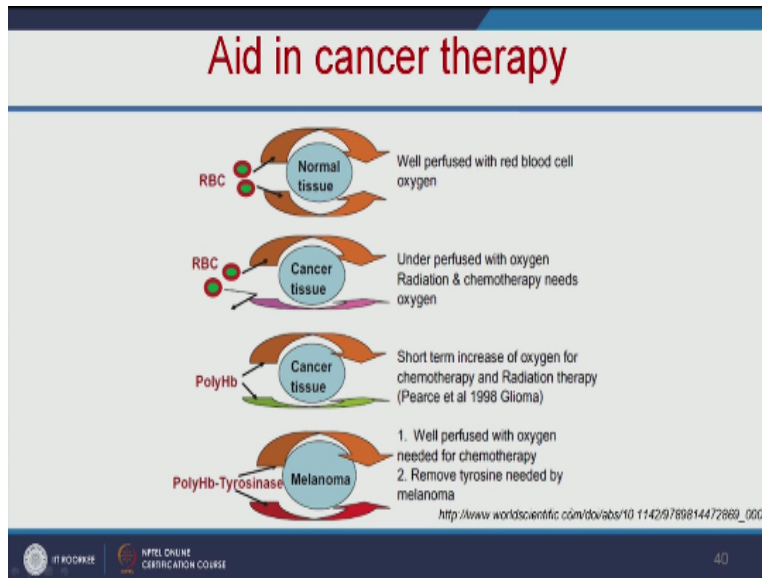
http://www.worldscientific.com/doi/abs/10.1142/9789814472869_0001

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So here we can use this artificial cells for Ischemia okay. So here when there is a arterial obstruction and that will narrow the artery so that will result in stroke and heart attack okay, so the normal RBC which is in the size of 7 to 8 m in diameter it cannot pass through this small passage okay and due to make of oxygen there is the high chance for getting this stroke as well as heart attack so when you use this poly hemoglobin solution it is in the size of nano so it can easily pass through this and it can supply the oxygen.

But the problem is so if there is a oxygen lake is prolong so due to reperfusion with an oxygen carrier it can release damage in oxygen radicals, so it may produce some oxygen radicals okay. So in this case we can use that poly hemoglobin with SOD and Catalase enzyme solution, so it can be supply the oxygen and also it will remove the oxygen radicals it is having dual function okay.

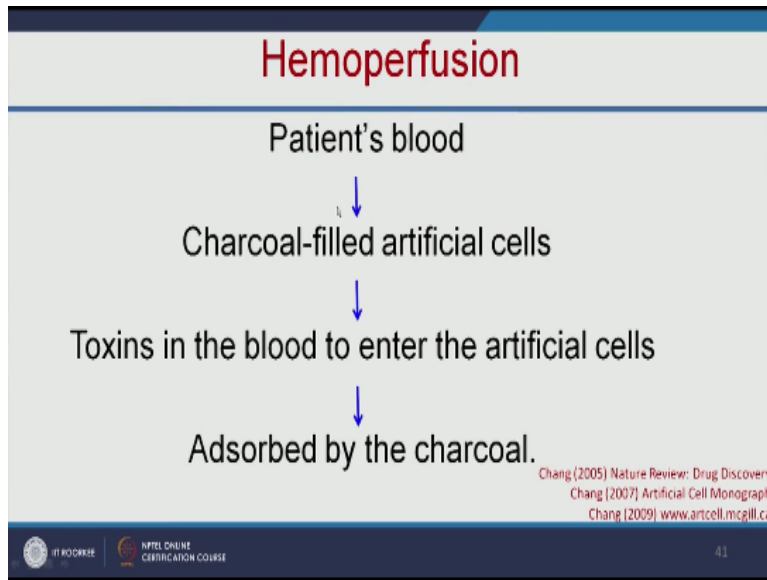
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So let see how we can sue this artificial RBC for cancer therapy so in the normal tissue so it is well perfused with RBC and oxygen but in case of cancer tissue it is under perfused with oxygen but for successful radiation chemotherapy you need oxygen so the normal RBC cannot enter ion to the cancerous tissue okay. So we can use the poly hemoglobin so due to small size it can easily enter the cancerous tissue, and it can increase the oxygen which could be useful for successful chemotherapy and radiation therapy.

And another thing is along with the poly hemoglobin we can also add a enzyme tyrosine's okay, so it will remove the tyrosine which is needed for the melanoma growth, so the first function is it will give the oxygen which is needed for chemotherapy and radiation therapy and it will also remove the tyrosine which is needed for melanoma growth. So in this way it is suppress in the cancer growth. So next thing is we can also use it for Hemoperfusion.

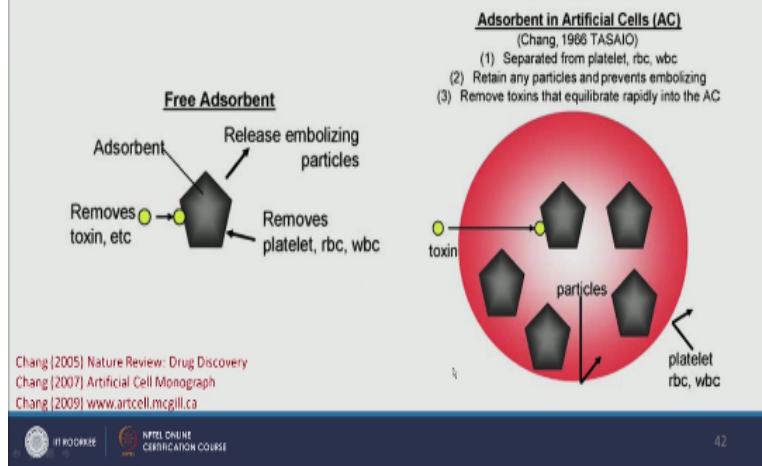
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So if there is a toxin in the patient blood that could be removed by charcoal filled artificial cells so the charcoal can be fill in the artificial cells so that toxins in the blood will enter the artificial cells and all the toxins will be absorbed by the charcoal inside the artificial cells.

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Hemoperfusion



So we can see here when you inject the free adsorbent what happens is it will remove the toxin but the problem is our platelet in RBC will go and attack it okay, so when you encapsulate in artificial cells it is separated from the platelet RBC and WBC and all the particles are inside the artificial cells so the toxins can enter inside and it can purify the blood.

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

Hemoperfusion

Millions of adsorbent artificial cells (70 grams) retained inside a small column (compared to an artificial kidney machine)

http://www.worldscientific.com/doi/suppl/10.1142/8776/suppl_file/8776_chap01.pdf

- A hemoperfusion device containing 70 grams of ultrathin membrane artificial cells containing activated charcoal (on the right side) is compared to the artificial kidney machine.



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So we can see here this one is millions of adsorbent material of artificial cells and the patient blood will pass through the artificial cells and the purified blood will be come to the patient and when compare to the artificial kidney machine we can see the smallest size so this hemoperfusion device containing 70g of ultrathin membrane artificial cells which containing activated charcoal and which could be the size could be compare with the artificial kidney machine okay.

And it can perform the removal of toxin from the blood so which can perform equivalent to this artificial kidney machine and it can remove all the toxin from your blood.

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Artificial cells as drug delivery vehicles

Drug (biodegradable membrane)

↓ fusion

Target tissue

↓

membrane degraded

Drug released

Chang (2005) Nature Review, Drug Discovery
Chang (2007) Artificial Cell Monograph
Chang (2009) www.artcell.mcgill.ca

And we can also use this artificial cells as a drug delivery vehicles so this artificial cells are made up of biodegradable membrane so we can add some kind of drugs or therapeutic molecules and this can be release at the particular location we can target this artificial cells to deliver the drug in to the particular location for various therapeutic applications.

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Artificial cells as biosensors

Artificial cells

↓



analytes

↓

signal

Coenzyme-depleted enzyme - glucose oxidase

Chang (2005) Nature Review: Drug Discovery
Chang (2007) Artificial Cell Monograph
Chang (2009) www.artcell.mcgill.ca


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And this artificial cells can also act like a biosensors so which will have some analyze okay so for example if there is a glucose level is more in the blood so it can interact with the glucose and it can produce some kind of flows and signal okay. So this artificial cell can also act like biosensors.

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Artificial Sperm


- Dr. Orly Lacham Kalpan succeeded in fertilizing a normal egg with an artificial sperm.
- Artificial Human Eggs Possible In few years



Biology

Scientists have created human sperm from skin cells. The researchers managed to reprogramme mature skin cells by introducing a cocktail of genes needed to create gametes and within a month the skin cell was transformed to become a germ cell, which can develop into sperm or an egg.

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 NPTEL ONLINE CERTIFICATION COURSE 46

And reason break through is this group they have made a artificial sperm okay so now it is a major problem is infertility okay so this research group they have made a artificial sperm and they have trying to make also artificial human eggs which could be possible in few years. So how they created so they have created the human sperm from skin cells and they reprogrammed this skin cells by introducing some genes and within a month the skin cell become a germ cell. So which can be developing in to sperm R and egg?

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Non medical use of artificial cells

- There are many developments and uses of the principle of artificial cells for agriculture, bioreactors, cosmetics, food production and aquatic culture (Poncelet and Neufeld, 2012).
- Others include, for instance, [the use of artificial cells for energy production by artificial photosynthesis](#) (Hammarstrom and Styring, 2008).

USEFUL WEBSITES ON ARTIFICIAL CELLS

<http://www.artcell.mcgill.ca>

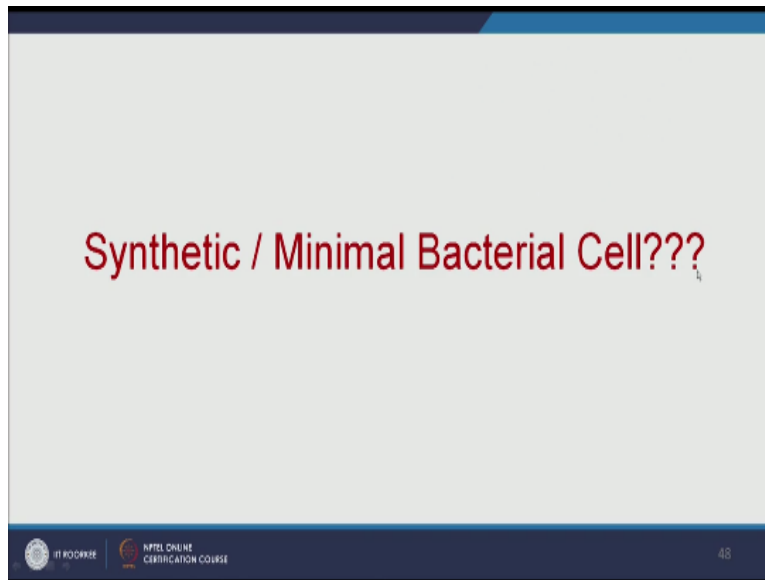
<http://www.artificialcell.info>

<http://www.artificialcell.org>



So this artificial not only for medical applications it can be also useful for non medical use for example it could be useful for cosmetics as well as food product and other application and also this artificial cells can also be useful for energy production by artificial photosynthesis and these are some of the useful websites if you want to get more information about the artificial cells, so before I conclude I want to discuss the another area that is synthetic or minimal bacteria cells.

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So this is different from the artificial cell so let us see what is synthetic or minimal bacterial cell.

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Synthetic / Minimal Bacterial Cell???

- **Synthetic cell:** as one that operates with a chemically synthesized genome
- **Minimal bacterial cell:** a bacterial cell to be minimal if it contains only the genes that are necessary and sufficient to ensure continuous growth under ideal laboratory conditions.

John Glass, The J. Craig Venter Institute, Rockville, MD and San Diego, CA



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So here the synthetic cell is a cell that operates with a chemically synthesized genome, and the minimal bacteria cell is a cell so it contains only the genes that are necessary and sufficient to ensure continuous growth under ideal laboratory conditions.

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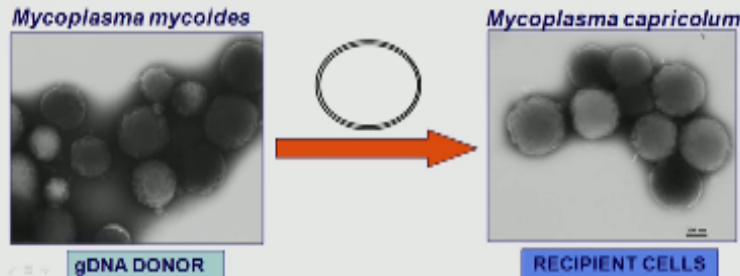
Genome Transplantation in Bacteria: Changing One Species to Another

Cecile Larigot, John I. Glass, Nina Alperovich, Kerben Flajer, Prashant P. Farnac, Clyde A. Hutchison III, Hamilton O. Smith, J. Craig Venter

As a step toward propagation of synthetic genomes, we completely replaced the genome of a bacterial cell with one from another species by transplanting a whole genome as naked DNA. Intact genomic DNA from *Mycoplasma mycoides* large colony (LC), virtually free of protein, was transplanted into *Mycoplasma capricolum* cells by polyanionic glycol-insulated transformation. Cells selected for

from incompatibility between the new genomes (6). Transplantation of nuclei as intact organelles into enucleated eggs is a well established procedure in vertebrates (7–9). Our choice of the term “genomic translocation” comes from the similarity to eukaryotic nuclear translocation in which one genome is cleanly replaced by another.

Genome transplantation is a requirement for the establishment of the new field of synthetic genomics. It may facilitate construction of useful microorganisms with the potential to solve pressing societal problems in energy production, environmental sustainability, and medicine. Chemically synthesized chromosomes must



So this article is published in science in 2007 okay so they have done the genome transplantation in bacteria so it will lead to changing from one species to another species so this kind of research was done by Craig Venter group okay so if the change in it from *Mycoplasma mycoides* to *Mycoplasma capricolum*.

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Science May 2010

RESEARCH ARTICLE

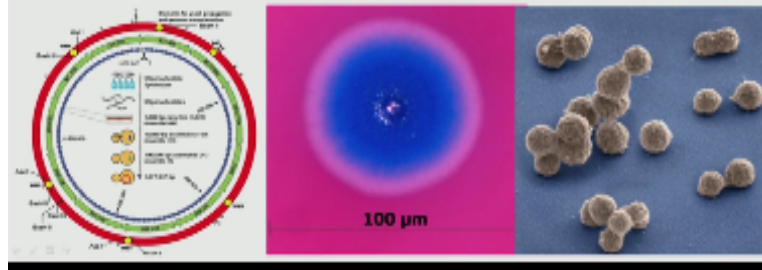
Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,¹ John I. Glass,¹ Caroli Lartigue,¹ Vladimír A. Nozka,¹ Ray-Yuan Chang,¹ Mickel A. Algira,² Geymed A. Benders,² Michael G. Montague,¹ Li Ma,² Marzia M. Moodie,² Chak Murtugudde,² Sergey Vashere,² Radika Krishnakumar,¹ Nancy Asad-Lorica,¹ Cynthia Andrews-Pfannkoch,¹ Eugene A. Dennis,¹ Lei Yeang,¹ Zhi-Qing Qi,¹ Thomas H. Segall-Shapiro,¹ Christopher H. Colwell,¹ Prashant P. Farnoc,¹ Clyde A. Hutchison III,¹ Hamilton O. Smith,¹ J. Craig Venter^{1,2*}

made *M. mycoides* or *M. capricornum* strains, or by simply disrupting the recipient cell's restriction system (R1).

We now have automated all of our previously established procedures and report the synthesis, assembly, cloning, and successful transformation of the 1.08-Mbp *M. mycoides* JCVI-syn1.0 genome, to create a new cell controlled by the synthetic genome.

Synthetic genome design. Design of the *M. mycoides* JCVI-syn1.0 genome was based on the highly accurate finished genome sequences of two laboratory strains of *M. mycoides* subspecies *capri* (GM17 (5, 11). One was the genome donor and



So there is another research published from the same group Craig Venter group okay so this as in science 2010, so they created a bacteria cell which could be control by a chemically synthesis genome. So let us see the details.

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Why to make a minimal cell?

- To define a minimal set of genetic functions essential for life under ideal laboratory conditions.
- To discover the set of genes of currently unknown function that are essential and to determine their functions.
- To have a simple system for whole cell modeling.
- To modularize the genes for each process in the cell (translation, replication, energy production, etc.) and to design a cell from those modules.
- To build more complex cells by adding new functional modules.




So why to make a minimal cell so to define a minimal set of genetic function essential for life under ideal laboratory condition and to discover the set of genes of currently unknown function that are essential and to determine their function and to have a simple system for whole cell modeling and to modularize the genes for each process in the cell okay. And to design a cell from those modules and to build more complex cells by adding new functional modules.

So the what are the idea is so we can make a minimal set of genetic functions okay which is essential for the survivor of the cell and we can also use there is a model system to understand if you have x gene what will be function if you have y gene what will be the function so it will act like a simple model system to understand the function of each and every gene.

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Minimal bacterial cell

- Researchers at the Craig Venter Institute chose to minimize *Mycoplasma mycoides* JCVI-syn1.0 the synthetic version of *Mycoplasma mycoides* because:
 - It has a small genome (1.08 MB).
 - It can be readily grown in the laboratory
 - We can routinely chemically synthesize its genome and clone it in yeast as a YCip
 - We can isolate the synthetic genome out yeast as naked DNA and bring it to life by transplanting it into a recipient mycoplasma cell.
 - They have developed a suite of tools to genetically engineer its genome.

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So for making the minimal bacterial cell researches at Craig Venter institute they have selected mycoplasma mycoides because it has a small genome which is only one MB okay and it can easily grown in the laboratory and we can easily synthesis genome and we can also isolate the synthetic genome from the yeast okay and they have several tools to genetically engineers genome. So that is why they have selected this mitcoplasma mycoids for making the minimal bacterial cell.

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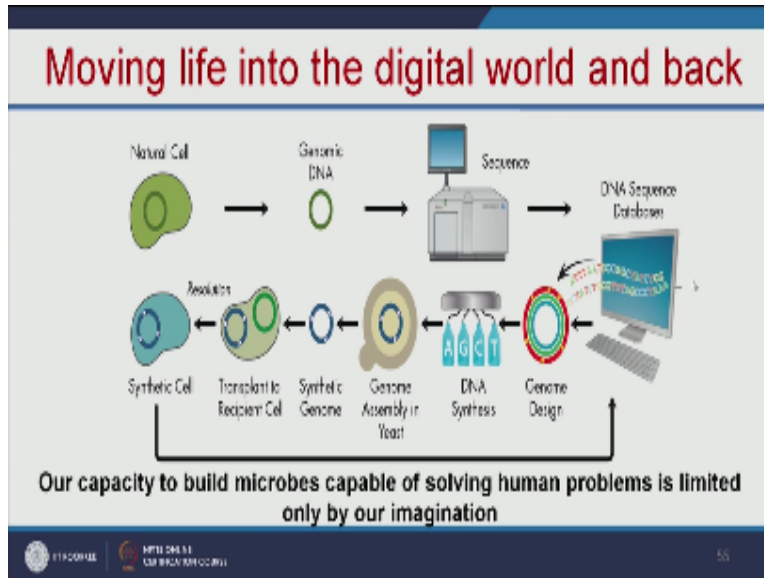
Starting point for minimization is the synthetic genome *M. mycoides* JCVI-syn1.0

- **TOP DOWN:** Start with the full size viable *M. mycoides* JCVI syn1.0 synthetic genome. Remove genes and clusters of genes one (or a few) at a time. At each step re-test for viability. Only proceed to the next step if the preceding construction is viable and the doubling time is approximately normal.
- **BOTTOM UP:** Make our best guess as to the genetic and functional composition of a minimal genome and then synthesize it.

So how to make this minimal bacterial cells there are two approaches one is top down approach and other one is bottom up approach okay so the top down approach is start with the full size viable bacteria then remove the genes and clusters of genes one at a time and that each step retest for viability and only proceed to next step if the presenting construction is viable and the doubling time is approximately normal.

So we can take the bacteria okay and remove a particular part of gen and just check it whether the bacteria is viable and the doubling time is normal so then only you can proceed to next step. So that is called as top down approach from the whole genome you are cutting down small, small pieces of genome or your are removing the small, small pieces of genome and you are making the minimal genome okay.

And next approach is bottom up approach okay so make our best guess to the genetic and functional composition minimal genome and then synthesis it, so the bottom up approach is a random approach so you can make a guess what are the genes required for the cell to be arrive okay so we can take those genes and we can synthesis those genes and put it in to the bacteria and we can check it whether the bacteria is arrive and how it is performing okay.
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So how they made the minimal cells or synthetic cells so they taken the natural cell and they take the genomic DNA and the sequent the genomic DNA and after the squinting they made the own genome design and the synthesis genome and the all the genomes are assemble in the yeast and they transfer to the recipient cell then the recipient cell genome was degraded then you will get the complete synthetic cell with the minimal genome. So let us see the possible future uses of this synthetic or engineer species.

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Possible future uses of synthetic & engineered species

- Increase basic understanding of life
- Increase the predictability of synthetic biological circuits
- Become a major source of energy
- Replace the petrol-chemical industry
- Enhance bioremediation
- Materials science – expand biology's use of the periodic table
- Drive antibiotic and vaccine discovery & production
- Gene therapy via stem cell engineering

So it will increase a basic understanding of life okay and also it will increasing the predictability of synthetic biological circuits and it will replace the petrol chemical industry it will make the bio fuels okay and it will enhance the bioremediation and it will also play major role in material science and it will drive the anti biotic and vaccine discovery and production and we can also use it for gene therapy via stem cell engineering okay.

So as a summary of this lecture, so in this lecture we have learnt what is artificial cell okay and how to make enzyme with artificial cells as well as a artificial RBC and what are the various applications of this artificial cells how it can be useful for cancer therapy and how it can be useful for enzyme therapy and we have also learnt what is synthetic cell and what is minimal bacterial cell and also its various applications. So I will end my lecture here I thank you all for listening this lecture I will see you in another interesting lecture.

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