İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

NANONETWORK MESSAGING TECHNIQUES AND DATABASE IMPLEMENTATIONS USING HUMORAL IMMUNITY

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Programme : Computer Engineering

JUNE 2010

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

NANOAĞ MESAJLAŞMA TEKNİKLERİ VE SALGISAL BAĞIŞIKLIĞA DAYALI VERİTABANI UYGULAMALARI

YÜKSEK LİSANS TEZİ

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HAZİRAN 2010

FOREWORD

I would like to express my deep appreciation and thanks for my advisor Prof. Dr. Sema Oktuğ. This work is supported by ITU Institute of Science and Technology.

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Alper Rasim Çakır

Computer Engineer

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ABBREVIATIONS

DNA	**:** Deoxyribonucleic acid
ssDNA	**:** Single stranded deoxyribonucleic acid
RNA	**:** Ribonucleic acid
mRNA	**:** Messenger ribonucleic acid
IgG	**:** Immunoglobulin G
IgM	**:** Immunoglobulin M
ATP	**:** Adenosine triphosphate
PHSC	**:** Pluripotent hematopoietic stem cell
ITAM	**:** Immunoreceptor tyrosine-based activation motif
MHC	**:** Major histocompatibility complex

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NANONETWORK MESSAGING TECHNIQUES AND DATABASE IMPLEMENTATIONS USING HUMORAL IMMUNITY

SUMMARY

Nano-machines as the basic functional units of nanonetworks can achieve very simple tasks due to their limited size and complexity. This drawback brings up the necessity of communication between nano-machines in order to achieve complicated tasks. In order to achieve communication between every nano-machine a unique identification mechanism, propogation and transmission systems are required. This work uses a nanonetworking schema based on the unique identification of ssDNAs attached to every nano-element. Propagation and transmission systems, which are also using ssDNAs on vesicles and microtubules, have avoided the disadvantages of short range communications based on ion spreading. Providing that host identification, propagation and transmission requirements are solved with a ssDNA based mechanism, the final necessity in the nanonetwork is a database mechanism for the nano-machines. Author of this thesis have proposed a humoral immunity based database mechanism which will be used in cells for storing the ssDNA addresses of the neighbour nano-elements. Besides the database implementation in biological environment, messaging framework which enables unicast, multicast and broadcast messaging among nano-elements, is also proposed in this work. Finally, the resulting nanonetwork environment is much closer to the information technology point of view with its ability of advanced messaging and database implementations. This framework could be used to employ nanonetwork implementations within daily life in next couple of decades.

NANOAĞ MESAJLAŞMA TEKNİKLERİ VE SALGISAL BAĞIŞIKLIĞA DAYALI VERİTABANI UYGULAMALARI

ÖZET

Nano-makinalar nanoağların en basit elemanları olarak kısıtlı kapasitesileri ve ebatları ile sadece çok basit görevleri yerine getirebilirler. Bu dezavantaj kompleks görevleri tamamlayabilmek için nano-makinalar arasında iletişim ihtiyacını da beraberinde getirmektedir. Nano-makinalar arasında ileşiminin sağlanabilmesi için her nano-makinanın kendine özel bir adresi ve bu adresleme ile uyumlu transmisyon ve yayılım mekanizmaları oluşturulmalıdır. Bu çalışma her nano-elemanın kendine özgü tek sarmal DNA adresinin olduğu bir nanonetwork altyapısı kullanmaktadır. Yayılım ve transmisyon sistemleri de bu tek sarmal DNA adresleme altyapısının kapsüller ve mikrotübüller üzerinde kullanımına dayanmaktadır. Bu şekilde biyolojik ortamlarda klasik yayılım ve transmisyon sistemi olan iyon dağılıma bağlı yöntemlerin dezavantajlarından kurtulunmuştur. Hücre adresleme, yayılım ve transmisyon sistemi ihtiyacının tek sarmal DNA'ya dayalı mekanizmalar ile çözümü sonrası, nanoağlarda son gereksinim nano-makinalar için bir veritabanıdır. Bu tezin yazarı veritabanı gerçeklemesi için hücrelerde salgısal bağışıklığa dayalı bir veritabanı sistemini önermektedir. Bu veritabanı sistemi yardımı ile hücler komşu hücrelerin tek sarmal DNA adreslerini saklayabileceklerdir. Bu çalışmada veritabanı uygulamalarının yanısıra nano-elemanlar arası yeni bir mesajlaşma altyapısı da önerilmiştir. Bu mesajlaşma altyapısı sayesinde nanonetworkdeki hücreler arasında tek yöne yayın(unicast), çoğa gönderim(multicast) ve tüm yönlere yayın(broadcast) mesajlaşma şekilleri mümkün olmuştur. Sonuç olarak önerilen bu nanoağ ortamı mesajlaşma ve veritabanı altyapıları ile bilgi teknolojilerin bakış açısına çok daha yakın bir altyapıya sahiptir. Bu altyapı önümüzdeki yıllarda nanonetwork uygulamalarının günlük yaşamda kullanımına olanak sağlayabilir.

1. INTRODUCTION

Nanotechnology can be defined as the science and engineering responsible from the design, synthesis, characterization and application of devices at nano-metre scale [1]. Nano-machines, which are capable of doing tasks such as sensing or simple computation, are the basic functional units at nano-scale in nanotechnology demonstrations. Although their potential encourages the nano-machine production, it is not easy to manufacture at nano-scale today. There are three approaches regarding the development of nano-machines. Top-down approach focuses on downscaling the existing micro-scale device components. Meanwhile, in bottom-up approach nano-machine development using individual molecules as the building blocks is proposed. Third approach is the bio-hybrid approach, which concentrates on the existing biological structures found in living organisms [2]. Since current manufacturing techniques do not allow building nano-machines efficiently, using existing biological structures is the painless way to start with. Nano-machines, both in biological systems or artificially created ones, can manage very simple tasks due to their limited size, complexity and mobility. Their ability to perform only simple tasks brings up the necessity of communication between nano-machines. Nanonetworking is defined as communication of the nano-machines with each other and sharing information among themselves in order to realize a common objective [3]. With the help of communication, they can cooperate and perform complex tasks [1]. Similar to the manufacturing techniques of nano-machines, regarding their communication method various techniques have also been proposed. Most promising method that can be used in nanonetworking is molecular communication. As an already used method in biology the basics of molecular communication has already been defined. However there are some drawbacks like communication radius of a sender or denaturalization of the ions. After all, design of the nano-machines and nanonetworking schema has various proposals each of which has its pros and cons.

This work has used a nanonetworking schema in which cells in a culture environment are employed as nano-machines. Culture environment overcomes the observation difficulty that can be faced in a living organism. Due to their free oscillation in the culture environment plasma cells are preferred in this work. Other types of cells (e.g. epithelium, liver etc.) are in the affinity of binding together tightly which would paralyze the nanonetworking environment. After providing the appropriate conditions for plasma cells in the culture environment, the next issue considered in this work is the short range molecular communication between the cells which includes host identification and propagation, transmission systems. In order to enable host identification with unique addressing schema this work uses the ssDNA attaching technique [4] over the cell membrane. ssDNA is also used on the vesicles and microtubules for controlled propagation and transmission. The communication mechanism used in this work has various advantages over the ion based communication. Most essentials of these advantages are the avoidance of communication radius problem and availability of unicast, multicast and broadcast messaging between the nano-machines (cells). To use the ssDNA mechanism without external intervention each nano-machine should have a database of other nano-machines' ssDNA addresses. This work has proposed a new database mechanism depending on the humoral immune system antibody creation technique on mammal plasma cells. Thanks to this mechanism every cell is able to remember the ssDNA addresses of its neighbours just like a plasma cell remembering previously encountered antigens. Hence each cell is able to communicate its neighbours directly with unicast, multicast or broadcast messaging. The proposed database mechanism in this work is also able to maintain its workflow on the ordinary cell lifecycle. A cell death does not block the system, because other cells are able to delete that cell from their database. Similarly, cell division does not also have an influence on the workflow of the nanonetworking schema providing that each new cell is artificially identified with a ssDNA.

This work is organised as follows. In Section 2, molecular communication including host identification based on ssDNA, propagation and transmission systems are discussed. In Section 3, the database mechanism based on humoral immunity of the mammals is handled. The effects of cell lifecycle and infrastructure for unicast,

multicast and broadcast messaging are also discussed in Section 3. Finally Section 4, concludes the thesis by giving future directions.

2. ISSUES REATED TO BIOLOGY

2.1 Objectives

This section aims to provide background information required to understand the details proposed in Section 3.

2.2 How Cells are Studied?

Cells are small and complex, that's why it is very hard to see and discover their structure and functionalities. The principal method that started cell biology is the light microscope, which became more advanced nowadays by use of beams of electrons and other forms of radiation. There are other types of microscopes that are used in cell biology, such as fluorescence microscope. Fluorescent molecules absorb light at one wavelength and emit at another. When these kinds of molecules are illuminated at its absorbing wavelength and then observed through a filter, they can be distinguished easily from other molecules [5]. In addition to advanced analysis with light and florescence microscope, today it is possible to have active intervention which is indeed much more efficient than passive observation for understanding complex procedures in cells. Active intervention helps in understanding how cells of different types can be separated from tissues and grown outside the body preserving their ordinary lifecycle [5].

2.3 Evolution and Classification of Cells

Living cells probably arose on earth about 3.5 billion years ago with the help of spontaneous reactions among molecules. The first cell like structures are assumed to be formed from simple biological molecules under prebiotic conditions. Today in laboratory environment it is possible to create small organic molecules from mixture of gases, water, electrical discharge or ultraviolet radiation. Evolution to today's complex creatures is believed to be triggered from the evolution of RNA molecules that could handle their replication. This capability triggered polypeptides synthesis. At the end of this evolution path, DNA double helix substituted RNA as a further steady molecule to store genetic information [5].

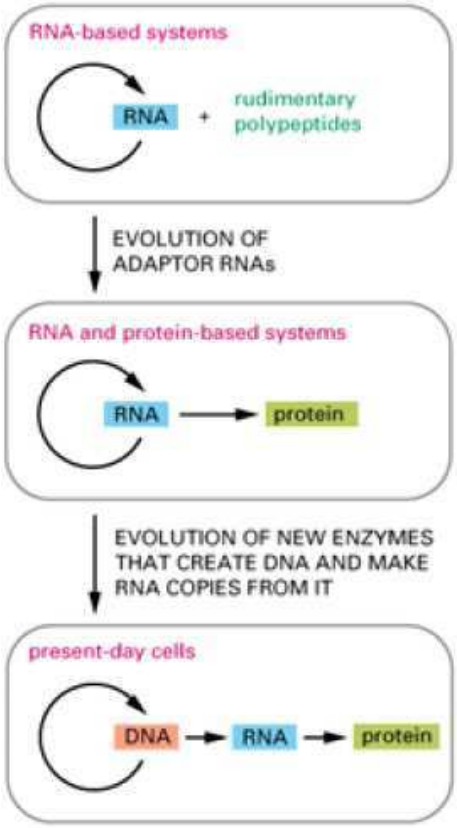

Figure 2.1 : Stages of evolution from RNA-based systems to present-day complex cell structures [5].

All plants, animals, and even micro organisms simply all living creatures are made up of cells. The simplest forms of life are solitary cells and they propagate by dividing into two. In much more complex organisms such as human beings groups of cells perform specialized functions. These cells and all living creatures on earth today are believed to have descended from a common ancestor cell which is formed

3 billion years ago. Differentiation is then caused by evolution and natural selection from this cell. 1.5 billion years later from the ancestor cell one of the most important steps in differentiation happened and eukaryotic cells are formed or differentiated from prokaryotic cells. Today these eukaryotic cells are found in animals and plants [5]. That's why cell formation and differentiation is crucial to maintain diversity. Today cells are divided into two main classes determined by the presence or absence of a nucleus. The ones without a nuclear envelope are called prokaryotic cells such as bacteria. However eukaryotic cells do have a nucleus which separates their genetic material from cytoplasm. Prokaryotic cells are much smaller in size, since they are very primitive compared to eukaryotic cells. Besides not having nucleus, the also do not have any cytoplasmic organelles or cytoskeleton. Prokaryotes are divided into two groups in the evolution, archaebacteria and eubacteria. Some archaebacteria live in extreme environments especially in primitive earth. For instance, thermoacidophiles can live in sulphur in high temperatures up to 80°C. Eubacteria does manage to live present day conditions in wide environments such as water, soil or human pathogens. The structure of a typical prokaryotic cell can be identified on Escherichia coli. The DNA of E.coli is a single circular molecule in nucleoid which is not surrounded by a membrane [6].

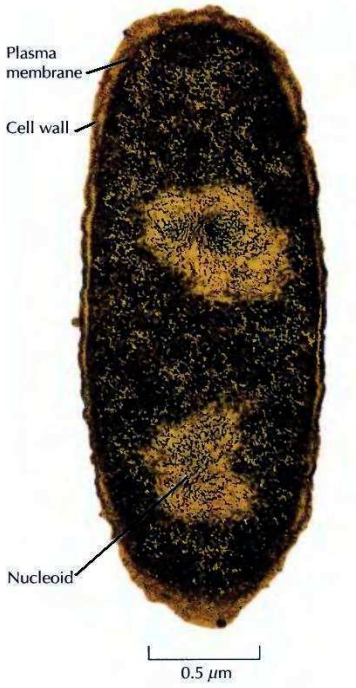

Figure 2.2 : Electron Micrograph of E.coli [6]

Eukaryotic cells are much more complex then prokaryotic cells. The nucleus containing the genetic material is the source for DNA replication and RNA synthesis. As well as nucleus, eukaryotic cells contain many organelles in which different metabolic activities are concentrated. This structure provided by the organelles provides efficiency in eukaryotic cells. These organelles have specific responsibility on the eukaryotic cell lifecycle. For instance, each eukaryotic cell needs a transport mechanism inside the cell in order to convey proteins to correct destinations. This mission is accomplished by golgi apparatus and endoplasmic reticulum. Meanwhile, mitochondria and chloroplast take place in energy metabolism. Mitochondrion is responsible for generation of energy (ATP) from oxidative metabolism. Whereas chloroplast takes role in photosynthesis in plant cells and green algae. Lysosomes are the organelles specialized in digestion of big (macro) molecules. Vacuoles perform similar objectives in plant cells with lysosomes. In addition to that it also takes place in storage facilities. Another internal organisation in eukaryotic cells is cytoskeleton which is a network of protein filaments extending throughout the cytoplasm. It helps on determining the cell shape and cytoplasm organisation. Movement of cells and organelle positioning is also done by cytoskeleton [6].

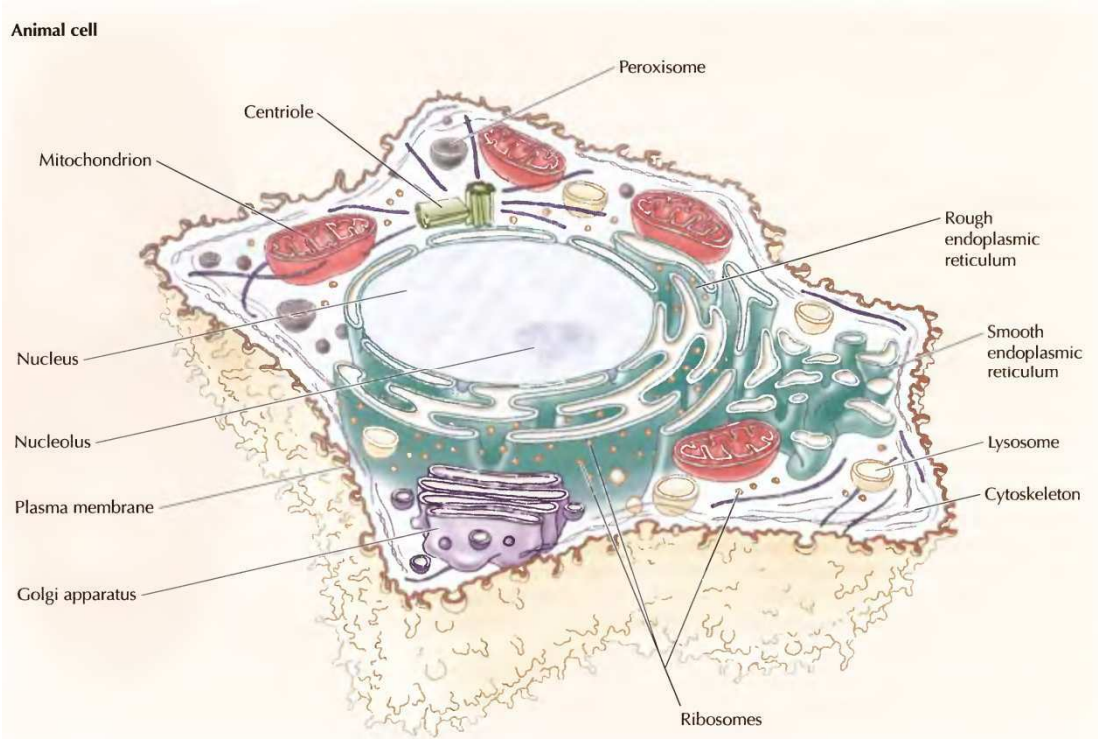

Figure 2.3 : Animal Cell [6]

2.4 Cell Specialization and Differentiation

Single cell organisms are very successful in adapting to variety of different environments. Today they constitute half of the biomass on earth. However, they do not have the ability to collaborate or division of labour. In the evolution, division of labor begins with multicellular organisms. The evolution to multicellular organisms started with colonies such as Volvox. In Volvox the individual cells form a colony by cytoplasmic bridges. Within Volvox colony there is some division of labour among cells. For instance, some of the cells are specialized for reproduction. These advantages of cell associations have guided the cells to multicellular organisms in the evolution path. Muticellularity has enabled a plant or animal to be large in size and have special parts of its body to be dedicated to some issues. These specialized parts of the body are serving to the whole of the organism. Such as, a tree has its roots to take water and minerals from the soil, and leaves in the air to capture sun light. At the end, multicellular organisms have two essential features: its cells become specialized, and they cooperate [5]. The most important step in specialization is the differentiation.

Cell differentiation is a special characteristic of cell growth and cell division. Cell differentiation is the changes in physical and functional properties of cells after the formation of embryo in order to form different parts and structures of the body. There are various studies about this topic but may be the easiest one to start with is an experiment done on a frog. In this experiment the nucleus of a mucosal cell of a frog is implanted into a frog ovum after removing the original nucleus. At the end, the experiment resulted in a normal frog which is a proof stating that even highly differentiated cells like mucosal cell carries all the genetic information for development of all other cells in the frog's body. This experiment proves that cell differentiation does not cause loss of genes but it represses different genes while leaving some others free to continue protein synthesis. These repressed genes can never function again. This causes human cells to produce a maximum of 8000 to 10000 proteins rather than potential 30000 or more if all of the genes were active [7].

Considering the differentiation of blood cells, they start their lifecycle in the bone marrow from a single type of cell, the pluripotential hematopoietic stem cell. All cells in the blood are derived from this stem cell.

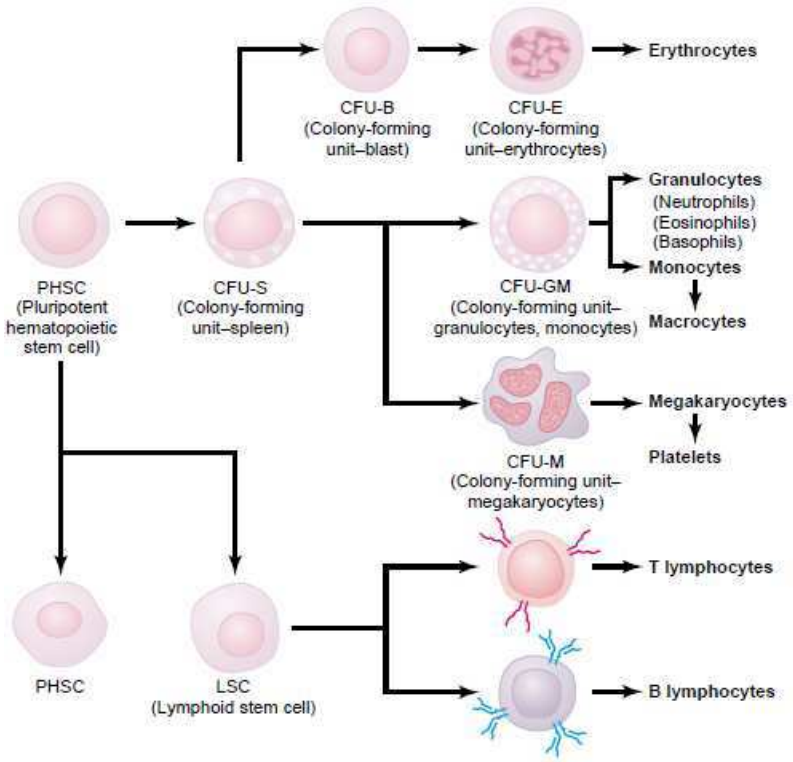

Figure 2.4 : Formation of different blood cells from the original pluripotent hematopoietic stem cell in the bone marrow [7]

As these cells reproduce from pluripotential hematopoietic stem cell (PHSC), some of them remain unchanged and they are stored in bone marrow to maintain a supply of these derived cells. However, the number of these PHSC's diminishes as individual gets older. Therefore if sterm cells are grown in culture they will be able to produce colonies of specific types of blood cells [7]. These kinds of cell differentiations enable various cells to get specialized in some functionality. In the evolutionary path of the human being, some of the blood cells concentrated on combating different infectious and toxic agents. This system with all of its actors is named as immune system.

2.5 Immune System

Human body is exposed to bacteria, viruses, fungi and parasites everyday. These kinds of attacks occur normally at different degrees in skin, mouth, respiratory passageways, intestinal track, lining membranes of the eyes and even the urinary tract. Human body is able to resist almost all types of organisms or toxins that tend to damage tissues or organs. This competence is called the immunity. Immunity is

composed of white blood cells (blood leukocytes) and tissue cells originated from leukocytes. This system fights against the infections either by destroying the invader (bacteria or virus) by phagocytosis or by forming antibodies and sensitized lymphocytes. Leukocytes are mobile units of the body's protective system. They are produced in bone marrow and lymph tissue. After production leukocytes are transported in the blood to different parts of the body. This transport mechanism does manage to send leukocytes to the areas of infection in order to strengthen the defence mechanism. In human body, there are six types of white blood cells. They are polymorphonuclear neutrophils, polymorphonuclear eosinophils, polymorphonuclear basophiles, monocytes, lymphocytes, and plasma cells. The white blood cells formed in the bone marrow are also stored within bone marrows until they are needed. When there is a need various factors cause them to be released. Normally, about three times as many white blood cells are stored in the bone marrow as circulate in the entire blood. This complex and valuable immune system can be analyzed in two parts in humans, acquired immunity and innate immunity. Acquired immunity does develop whenever the body is attacked by a bacterium, virus or toxin. The acquired immunity does usually need a time frame of weeks or sometimes months in some cases to be able to fight against the attacker. On the other hand innate immunity is available on the humans starting from the first day of the life. These two immune system responses attack together to inactivate or destroy the invader [7].

2.5.1 Innate Immunity

Innate immunity results from general processes rather than processes caused by particular disease organisms. Therefore, it protects the body in a non-specific manner regardless of the invader. Innate immunity consists of many structures and processes in the body [7]. It mainly includes the following

- Phagocytosis of bacteria and other invaders by white blood cells and cells of the macrophage system.
- Destruction of swallowed organism by the digestive enzymes and acid secretions of the stomach.
- Resistance provided by the skin to organism invasions.

- The chemical compounds such as lysosome, basic polypeptides, complement complex or natural killer lymphocytes are able connect foreign organism and toxins in order to destroy them.

2.5.1.1 Phagocytosis

Phagocytosis is an evolutionarily conserved process utilized by many cells to ingest microbial pathogens [8]. Phagocytes must be selective of the material that is phagocytized. Otherwise normal cells and structures of the body might be attacked. The decision process depends on three selective procedures. First of all, the natural structures in tissues resist phagocytosis with their smooth surfaces. Secondly, the natural substances of the body have protective protein coats that deny phagocytes. Dead tissues and foreign particles do not have this protective coat. Thirdly, the antibodies produced by the immune system against infectious agents, adheres to their membranes and this process makes the agent vulnerable to phagocytosis. Phagocytosis is achieved by neutrophils and macrophages.

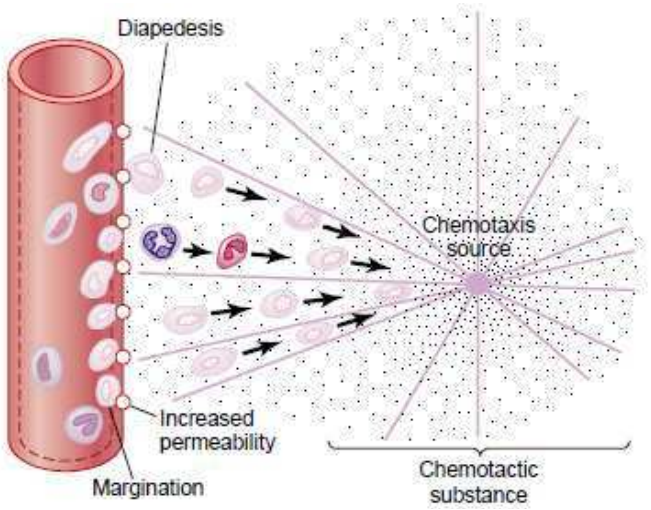

Figure 2.5 : Movement of neutrophils by diapedesis through capillary pores and by chemotaxis toward an area of tissue damage [7]

Neutrophils can attack and destroy an invader (i.e. bacteria) even in the circulating blood. Neutropils and macrophages can also move through tissues by ameboid motion which is triggered by a chemical. Many substances in tissues cause both neutrophils and macrophages to move toward the source of this chemical. This is also known as chemotaxis. The concentration is massive around the source of infection. This directs the unidirectional movement of the white cells. Once the foreign particle has been phagocytized, lysosomes and other cytoplasmic granules in

neutrophil or macrophage immediately contacts with the phagocytic vesicle in order to digest the phagocytized particle [7].

Innate immunity helps the human body to be resistant to such diseases as paralytic viral infections of animals such as hog cholera, cattle plague etc. On the contrary, many animals are also resistant or immune to human diseases such as human cholera, measles etc. which might be even deadly to human beings [7].

2.5.2 Adaptive (Acquired) Immunity

Human body is able to develop extremely powerful immunity against specific invaders such as lethal bacteria, viruses or toxins. This process is generally called acquired or adaptive immunity. Adaptive immunity is provided by special immune system that forms antibodies and/or activated lymphocytes which are able to attack and destroy the particular invading organism or toxin. Adaptive immunity is able to protect human body even in extreme conditions. For example, human body can be protected from certain toxins such as paralytic botulinum in very high doses, 0,00001% of which would be lethal on a body without the protection of the immune system. This issue emphasizes the importance of immunization process on human beings against diseases and toxins [7]. Two types of acquired immunity are available in the human body. The first one depends on the antibodies that are circulating in the blood plasma. Antibodies are capable of attacking the invading agents. This is called humoral immunity. Second type of adaptive immunity is called cellular immunity which is structured by the T lymphocytes. T cells are produced in lymph in order to destroy the foreign agents [9].

After origination in bone marrow, lymphocytes migrate to thymus gland where they divide in order to increase in size and diversity. The diversity of lymphocytes gives them the ability to react different specific antigens. Hence, each lymphocyte develops specific reactions in order to block specific antigens. Due to this mechanism diversity increases to thousands of different types in lymphocytes. After the production, these lymphocytes leave the thymus and spread through all body via blood. During the production in thymus, it is crucial to maintain that these lymphocytes will not react against any protein of tissues of body itself. If not the lymphocytes would be deadly to body itself. Thymus selects the ones that should be send out to entire body via blood and the ones that should be destroyed.

Lymphocytes are very sensitive; they even react to the transferred tissues from another person, especially in transplantation [7].

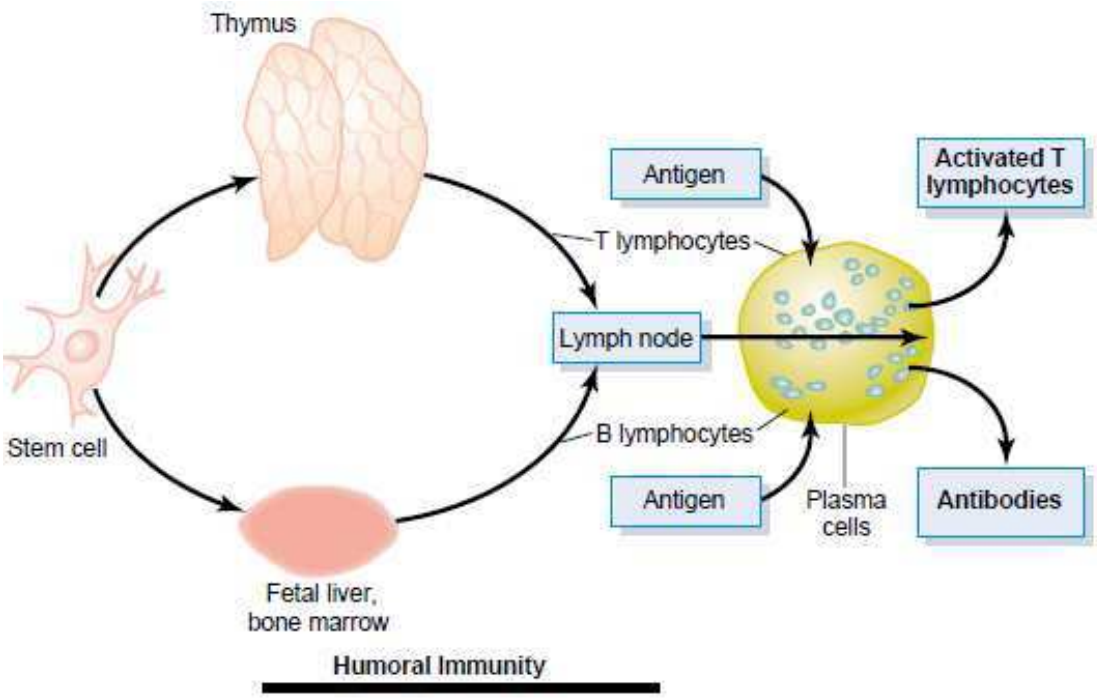

Figure 2.6 : Formation of antibodies and sensitized lymphocytes by lymph [7]

A human being is able to produce more than 10^8 distinct antibodies and more than 10^{12} T cell receptors. Each of these antibodies and T cell receptors forms a different surface for specific bindings of the invaders. The crucial point in the defence mechanism of human body is this diversity. If 40000 genes in the human genome is taken into account, the power of the diversity resulting in 10^8 distinct antibodies and more than 10^{12} T cell receptors, would more obvious [9].

2.5.2.1 Humoral Immunity

In humoral immune response, functional proteins called antibodies or immunoglobins takes place. They function as recognition elements that bind to foreign molecules and serve as markers signalling invasion. A foreign molecule that selectively binds to an antibody is called an antigen. Immunoglobin G (IgG) is the major antibody in the serum. IgG consists of three fragments two of which are used to bind antigens. These fragments are called F_{ab} (fragment for antigen binding). The other fragment is called F_c, it does not bind any antigen but it has other significant biological activities such as initiation of the complement cascade, a process that leads

to break down of the target cells. IgG contains two kinds of polypeptide chains, L (light) and H (heavy) chain. Each L chain is linked to an H chain and H chains are linked to each other [9].

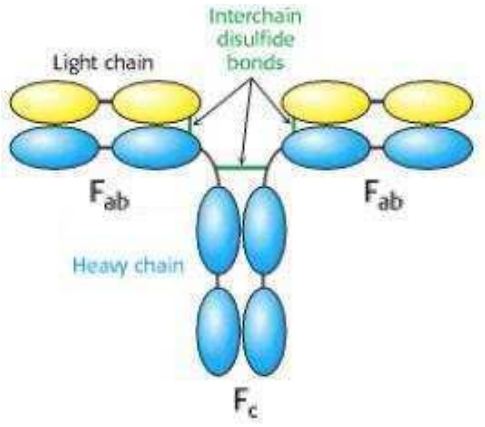

Figure 2.7 : Schematic view of an IgG molecule [9]

Each F_{ab} contains an antigen binding site. Having two F_{ab} fragments, IgG has the ability to cross-link multiple antigens. In addition to that the flexible polypeptide regions between F_{ab} and F_c allow variation in the angles between F_{ab} units in wide range [9].

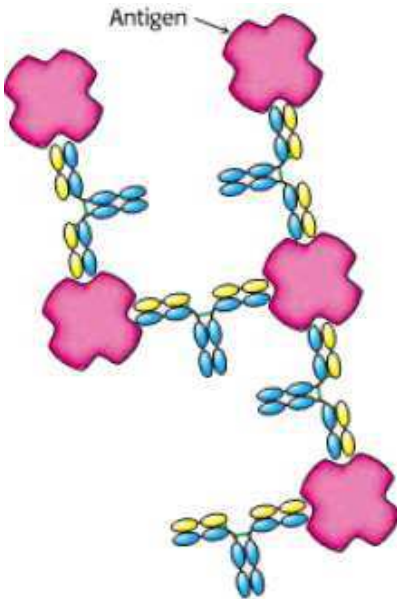

Figure 2.8 : Two binding sites allows cross-link of multivalent antigens such as viral surfaces [9]

A comparison of the amino acid sequences of different IgG antibodies from different mamals reveals that there are very similar parts in different IgG antibodies. The remaining parts make the diversity possible in antibodies. The amino-terminal

immunglobulin domain of the chain is referred as the variable region. Remaining domains from the variable region are called the constant regions. The variable domains are named as V_L and V_H, the remaining constant domains are C_L1, C_H1, C_H2 and C_H3. The figure below shows the corresponding places in the IgG antibody [9].

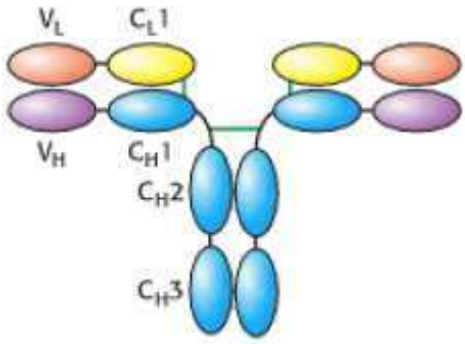

Figure 2.9 : Variable and constant regions [9]

The binding of antigens to antibodies is governed by the principal similar to enzyme-substrate bindings. The opposition of complementary shapes results in various contacts between the amino acids at the binding surfaces of the molecules. Hydrogen bonds, van der Waals interactions and electrostatic interactions are used in corporation to have specific and strong bindings [9].

Not only human beings, but all mammals are able to synthesize huge amounts of specific antibody against any foreign thread within a matter of days, after being exposed to it. Antibody diversity is maintained by the amino acid sequences in the variable regions of heavy and light chains. In 1965, Dreyer W. and Bennett C. proposed the diversity mechanism of antibodies, in which multiple variable genes (V) are separate from a single constant (C) gene in embryonic DNA. According to the proposed model different V genes are joined to C gene in order to maintain the diversity of antibodies. When isolation of pure IgG mRNA is managed twenty years later then this hypothesis, it is proved that immunoglobulin genes are rearranged in differentiation. Besides V and C genes there are J (joining) genes located near the C gene in embryonic cells. In the differentiation of an antibody production, a V gene becomes merged to a J gene in order to complete the variable region of the gene. A single V gene is linked to a J gene to form a VJ region. This V and J genes are selected randomly and the place of the joint between them is also random. Therefore, many VJ combinations are generated by this randomness. Randomly formed VJ region is then joint with the constant gene (C), resulting in a VJC gene sequence.

Then from this rearranged gene, pre-mRNA is produced by transcription. After transcription splicing is applied to have the mRNA which will then be used in translation and processing. After all these steps the result is an L chain protein [9].

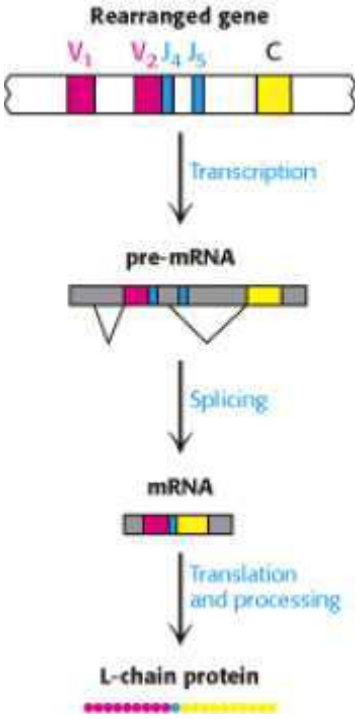

Figure 2.10 : Light chain expression [9]

V and J genes are important contributors to antibody diversity. In the variable gene formations of L chain protein, 40 V genes and 5 J genes takes place. Recombination of these VJ genes strengthens the diversity between these genes [9].

In variable domains of heavy chains, V_H genes encode residues 1 to 94 and J_H segments encode residues 98 to 113. From 95 to 97 it is called the diversity segments (D). In total 27 D segments is available between 51 V_H and 6 J_H segments. The recombination process in heavy chains first joins a D segment to J_H segment, after that V_H segment will be joined to DJ_H. Since there are three gene segments available in H chains, there is more diversity than the L chains with two gene segments. Random formation of these gene segments is handled by the specific enzymes in the immune system. These proteins are called RAG-1 and RAG-2 [9].

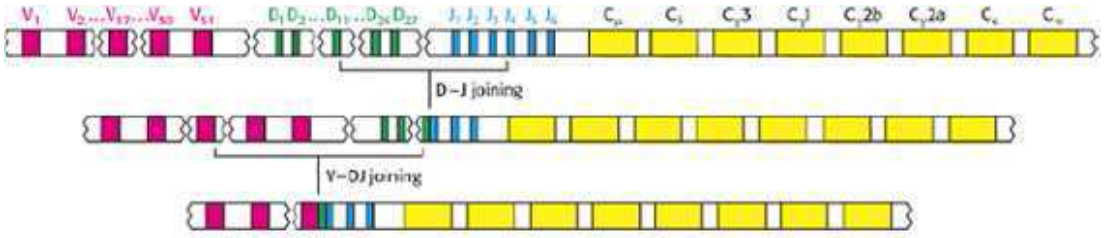

Figure 2.11 : V(D)J Recombination [9]

Consequently, the chains have the diversity maintained by these gene segments. For the light chain, there are about 40 V-segment genes and 5 J-segment genes. This brings up a total of 40*5=200 possible light chain formations from the combinations of V and J segment genes. In addition to these light chains, 120 λ light chains can be generated. In the heavy chains, involving segment genes are 51 V, 27 D and 6 J. Hence, the number of complete V_H genes that can be formed is 8262. The association of these possible arrangements of gene segments yields to 2,6 $x10^6$ different antibody formations. In addition to these formations, points of segment joining and other mechanisms increase this value by at least two orders of magnitude. More diversity in antibody chains is brought in by somatic mutation. These mutations are applied into the recombined genes. With the help of this process antibodies that do fit more precisely into antigens can be selected. At last, there are three sources of diversity which are germ-line, somatic recombination and somatic mutation [9].

Consequently, in the aim generating of immune response, the first step is highly diverse antibody molecules. The next stage is selection of particular set of antibodies functioning against a specific invader. This selection occurs on immature B cells in the bone marrow [9]. Starting from the fetal development, lymphocytes come from the bone marrow. The lymphocytes in thymus are transformed into T lymphocytes. B lymphocyte transformation occurs in bursal equivalents which are fetal liver and bone marrow [10].

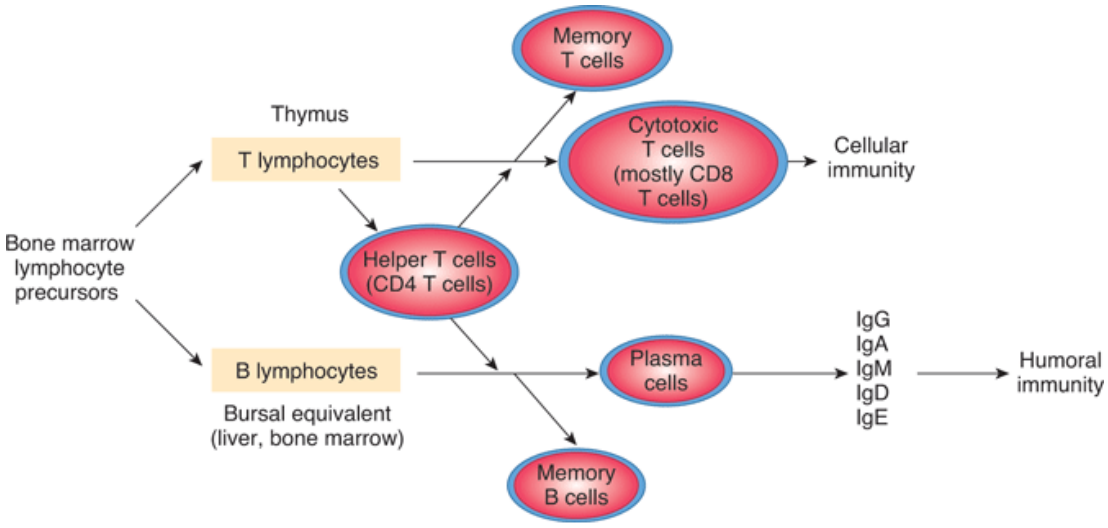

Figure 2.12 : Development of Acquired Immunity [10]

These B cells have a receptor complex that consists of a membrane-bound IgM molecule. IgM is bound to two Ig-α-Ig-ß. The amino acid termination of each protein is at the outside of the cell and they correspond to a single immunoglobin. The carboxyl termination inside the cell has 18 amino acids which are called immunoreceptor tyrosine-based activation motif (ITAM). Each of the B cells expresses around 10^5 IgM's all of which are identical in amino acid sequence. This means they have the same antigen binding specificity. These antigen bindings will trigger the growth of the antibodies that are effective specifically on that antigen. Hence, antigen-immature B cell binding triggers differentiation of a particular immature B cell and production of an antibody which has unique specificity. This differentiation process begins with the antigen antibody binding on the membrane and signalling process continues downstream to activate gene expression, which shows the way to the B-cell differentiation and motivation of cell growth. Triggering a differentiation process with a membrane protein have significant advantages considering the fact that the surface of many viruses, bacteria and parasites are characterized by the arrays of identical membrane proteins or membrane associated carbohydrates. This differentiation generates IgM antibody which is the first class of antibody to appear in the serum after exposure to an antigen. The presence of 10 combining sites permits IgM to bind tightly to antigens containing multiple epitopes. These 10 combining sites in IgM compared to 2 sites in IgG is brings a big plus in binding many antigens which might escape from IgG. Besides these benefits, B-cell differentiation might have same drawbacks especially on tissue, organ

transplantation. Transplanted tissue triggers a wide range of antigens, which causes the immune system to reject the new tissue or organ. In these kinds of cases drugs like cyclosporine, a powerful suppressor of immune system blocks this process [9].

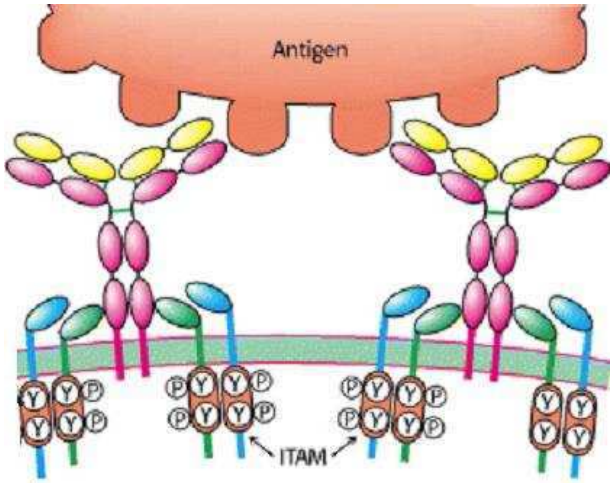

Figure 2.13 : B-cell Activation [9]

2.5.2.2 Cellular Immunity

In cellular immune system, T lymphocytes namely killer T cells kill the cells that display foreign motifs on their surfaces. The cellular immune system response is depending on the specific receptors on the surfaces of the T cells. Antibodies in the humoral immune system are also highly effective against extracellular pathogens, but they are not very successful against micro organisms that are predominantly intracellular like mycobacterium or viruses. Antibodies are not very effective on these kinds of pathogens because they are shielded by the host-cell membrane. In order to protect human body from these intracellular pathogens, cell mediated immunity did evolve. T cells continually scan the surface of all the cells and kill the ones that show signs of foreign motifs. This task is not very simple since intracellular organisms are not very willing to leave traces on the surface of their hosts. In this sense, the more pathogens are successful in hiding themselves the more it gets difficult to detect them. In order to overcome this problem, vertebrates have devised a mechanism called cut and display in order to expose the presence of the intruders. Most of the vertebrate cells display on their surfaces a sample of peptides derived from the digestion of proteins in their cytosol. These peptides are put on view by the integral membrane proteins that are encoded by major histocompatibility complex (MHC). The presentation of these peptides in the plasma membrane starts in cytosol with the degration of proteins, both self proteins and pathogen proteins. Digestion is

carried out by the proteasomes and the resulting peptide fragments are transported from the cytosol into the endoplasmic reticulum by an ATP pump. In the endoplasmic reticulum, peptides combine with MHC proteins, and the forming complexes are targeted to the plasma membrane [9].

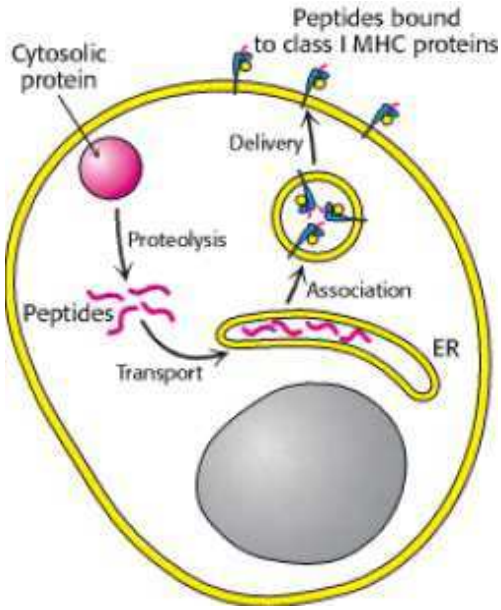

Figure 2.14 : Presentation of peptides from Cytosolic proteins [9]

Foreign peptides are bound to MHC proteins means that the cell is infected. This peptide will be used as a mark for destruction by T cells. Foreign peptide-MHC complex, the T cell receptor and accessory proteins triggers a flow that provokes the apoptosis in the infected cell. Hence, the infected cell is not killed directly but it is triggered to commit suicide [9].

The T cell receptor consists of a 43-kd α chain (T_α) joined by a disulfide bond to a 43-kd β chain (T_β). T_α and T_β are like L and H chains of immunoglobulin, they consist of variable and constant regions. These domains of the T cell receptor are homologous to the V and C domains of the immunoglobulins. The genetic architecture of these proteins is also similar to the immunoglobulins. The variable region in T_α has 50 V and 70 J segment genes. T_β is encoded by 2 D, 57 V and 13 J segment genes. Diversity of component genes and joining modes of them increase the number of distinct proteins formed. Therefore, 10^{12} different T cell receptors could arise from the different combinations of genes. This enables the T cell receptors to recognize very large number of different epitopes. All of the receptors on a T cell have the same specificity. The variable regions on the α and β chains of

the T cell receptors form a binding site which recognizes the epitope, foreign peptide bound to MHC protein. In order to have a binding with T-cell receptor, foreign peptide and MHC protein should be together, they do not bind to receptor solitary [9].

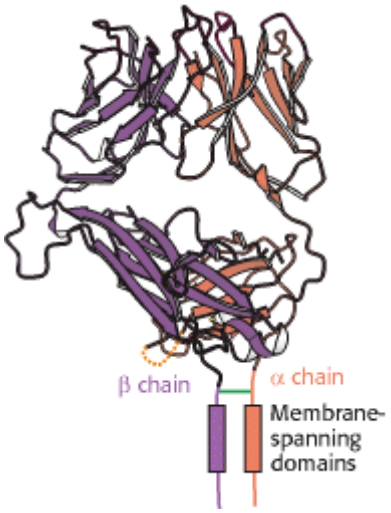

Figure 2.15 : T Cell Receptor [9]

The T cell receptor does not work alone in recognizing the target cells. Cytotoxic T cells articulate a protein called CD8 (cluster of differentiation 8) on their surfaces. This protein is fundamental in detection of MHC peptide complex. Antibodies specific for a particular cluster differentiation protein are very important in following the development of leukocytes and in determining new interactions between specific cell types [9].

There are several types of T cells, which can be divided into three groups. First group is the helper T cells. This kind of T cells constitute more than three quarters of them. As their name implies, they help other immune system cells in their functionalities. They function as regulator of immune system functions. They do this by structuring a series of protein mediators which are called lymphokines. These mediators have impact on other immune system cells. They are very crucial in immune system work flow, and in the absence of the lymphokines from the helper T cells, the immune system is almost paralyzed. In fact, it is the helper T cells that are inactivated by the AIDS (acquired immunodeficiency syndrome). The second group of is cytotoxic T cells. They are the direct attack cells which are capable of killing microorganisms. They sometimes also kill the body's own cell, that's why they are also called killer T cells. The receptors proteins of this kind of cells allows them to bind on the organism

or cells that contain the binding specific antigen. After binding, they kill the attacked cell [7].

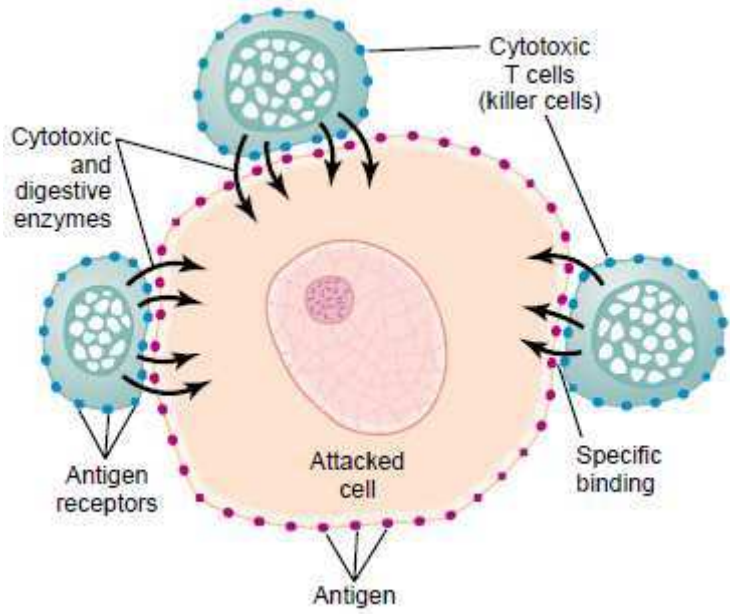

Figure 2.16 : Direct destruction of an invading cell by cytotoxic T cells [7]

Third group is suppressor T cells. They are capable of suppressing the functions of the cytotoxic and helper T cells. This group of T cells aims to prevent the cytotoxic cells from causing damage to the body's own tissues [7].

2.6 Cell Cycle

All cells arise by division of the existing cells, with this logic every cell living on earth today is thought to be descended from a single ancestor cell that lived 3 or 4 billion years ago. Throughout this period, cells and organisms evolved and this evolution has depended on the transmission of genetic information by cell division. Therefore cell reproduction is a fundamental issue for living creatures. In the development of multicellular organisms, a single cell divides and transforms into several communities of cells those results in diverse tissues and organs. Cells might also be dividing in order to replace the neighbour cells which might be dead as a result of a natural cause or an environmental damage. Such a complex thing like cell does divide following a complex series of events which is divided into a series of events in order to simplify the process. Mainly, first the duplication of the cell's contents is achieved and later the division takes place resulting into a pair of daughter cells. In the first part of this cell cycle, cytoplasmic organelles, membrane,

structural proteins and RNA's are replicated which as a result doubles the cell size. The chromosomes should also be divided, and this is achieved in a separate stage called S (synthetic) phase. Finally, the distribution of these duplicated components into individual daughter cells occurs in another stage called as M (mitotic) phase. This series of events in total is called the cell cycle. The duplication and division of cellular components must be achieved with extreme precision. This precision is achieved with the enzymes and regulatory mechanisms that ensure the events of cell cycle occur in the correct order. This enzymes and regulatory mechanisms stipulate that distribution of chromosomes is done after they are duplicated, or division into daughter cells begins after organelles are duplicated. In order to administer cell cycle phases and transitions between them, cell cycle control system has evolved in eukaryotic cells. This control system is used to control the switch on the cell cycle events at the correct time and in the correct order. The programming of this control system is prepared by the dependence of one event on another, which blocks premature events to be executed [11]. This control mechanism inside the cell is also used in multicellular organisms in order to control the division rate of specific cells. The cells of all tissues in the human body must grow in a coordinated fashion. Some cells, including most neurons, do not divide after birth. In contrast, the intestine is constantly shedding the cells; therefore new intestinal cells are regenerated each day. The mitosis division rate of the tissue cells are carefully regulated by various hormonal activities in the body. If there is a problem in these hormonal activities, the mitosis rate will be out of control. As a result fewer cells might be generated than they are sloughed off, which will cause the tissue to be non-functional. In another case more cells can be generated than they are sloughed off and this might cause tumorous outgrowths [12].

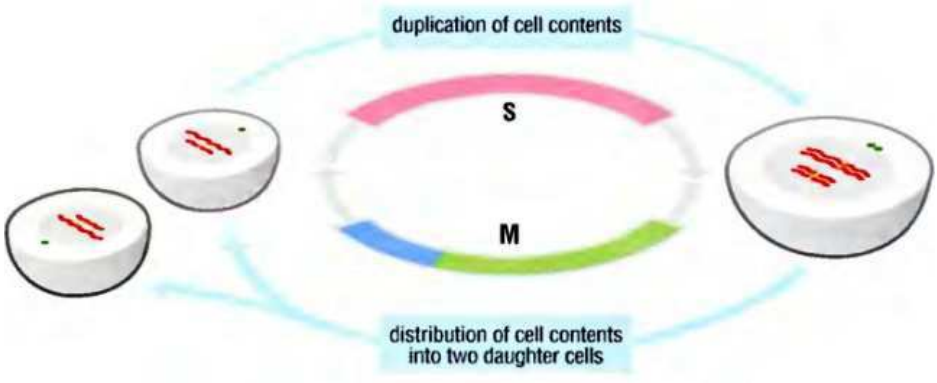

Figure 2.17 : The Cell Cycle [11]

If details of the cell cycle is analysed, it can be seen that the stages of eukaryotic cell cycle are based on the chromosomal events. Early in the cycle, DNA is replicated and chromosomes are duplicated in the S phase. In this phase DNA double helix formation is opened and DNA synthesis starts to copy the DNA strands. The second major phase in the cell cycle is the M phase, in which nuclear division (mitosis) and cell division (cytokinesis) occurs. After M phase, the cell is in interphase period till the beginning of the next phase [11].

Mitosis is a complex process that distributes the duplicated chromosomes equally into daughter nuclei. In early mitosis the sister chromatids are attached to the mitotic spindle, bipolar array of proteins polymers called microtubules. By the midpoint of mitosis (metaphase), sister chromatids are attached to microtubules which are coming from the opposite poles of the spindle. The next phase is anaphase in which sister chromatid cohesion is destroyed and sister chromatids are separated from each other (sister chromatid separation). This separation is achieved by the microtubules of the spindle pulling the separated sisters to opposite ends of the cell. This action is called sister chromatid segregation. At the end of these phases two sets of chromosomes are packaged into new daughter nuclei. After the mitosis, the cell divides by cytokinesis. This process is mainly the deposition of new plasma membrane, and new cell wall. The cell cycle is completed when the original cell is finalized into two cells of the same type [11].

There are additional phases which are common in most eukaryotic cells. These are known as gap phases between S and M. Gap phases provide additional time for the cell growth which takes much more time then duplication and segregation of chromosomes. G1 gap phase occurs before the S phase, and G2 occurs before M phase. G1 is the important period where the cell decides if it will continue to the cell division or exit the cell cycle. If the cell is in unfavourable growth conditions or inhibitory signals from other cells are received, cells may pause in G1 or even switch to a nondividing state which is called G0. In human body many of the cells are in nondividing state, and they are differentiated which disables them to re-enter the cell cycle [11].

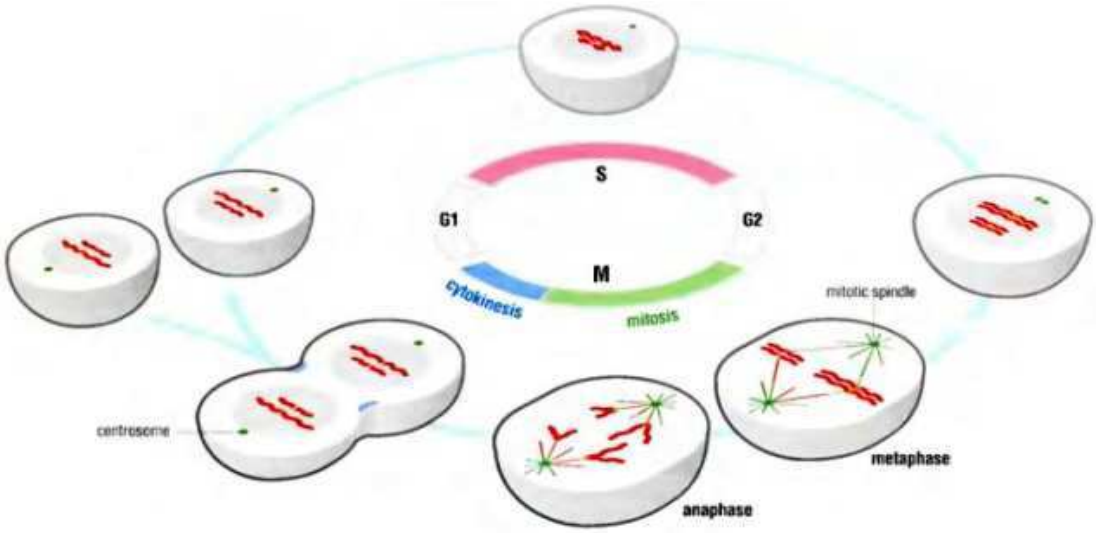

Figure 2.18 : Eukaryotic Cell Cycle Phases [11]

Cancer as a cell cycle disease is the centre of interest in this area. Understanding the principles of the cell cycle is the key to understanding the pathogenesis of the cancer but also of other diseases with imbalanced production. Along with cancer treatment, cell cycle is one of the most important parameters that define the response of a body against a particular drug. Cell cycle specific therapy for the disorders is very important in case of diseases like Alzheimer [13].

2.7 Nanotechnology and Nanonetworks

Nanotechnology is a multidisciplinary field that covers various devices which are drawn from physics, biology, chemistry or engineering. It can be defined as the science and engineering which is in charge of design and application of devices whose smallest functional unit is on the nano-meter scale [14]. This basic functional unit in nanotechnology is called nano-machine which is composed of arranged set of molecules. Nanotechnology has a high potential for several applications in biomedical, industrial and military fields [2]. However, it depends on the investigations at manufacturing tecniques at very small scale. Due to this dependence on manufacturing skills, nanotechnology has to wait for the advanced technologic background in this area. During this period there have been large numbers of experiments and computer simulations targeting these kinds of small scaled devices [15]. Nowadays, nanotechnology is stimulated by technological improvements in several disciplines some of which are development of ultra-precision process control

systems which can achieve nano-metre tolerance surfaces; ability to manipulate and assemble individual molecules and atoms; advances in mathematical modelling techniques which are used to predict mechanical and chemical properties of nano-machines [16]. These improvements in science might enable nano-scale machines to be used on new solutions for the existing problems in the near future.

2.7.1 Nano-machines and their components

This first issue that needs to be considered is the nano-machine production. Nano-machine is a device which is able to perform simple tasks at nano-level such as communicating, data storing, sensing or computing. There are three different approaches for the development of nano-machines. These approaches are top-down, bottom-up and bio-hybrid. In the top-down approach, nano-machines are developed by downscaling current microelectronic technologies. However, in bottom-up approach nano-machines are designed starting from the molecular components which assemble themselves chemically using the principles of molecular recognition. The third approach is bio-hybrid which supports the use of existing biological nano-machines such as molecular motors [2].

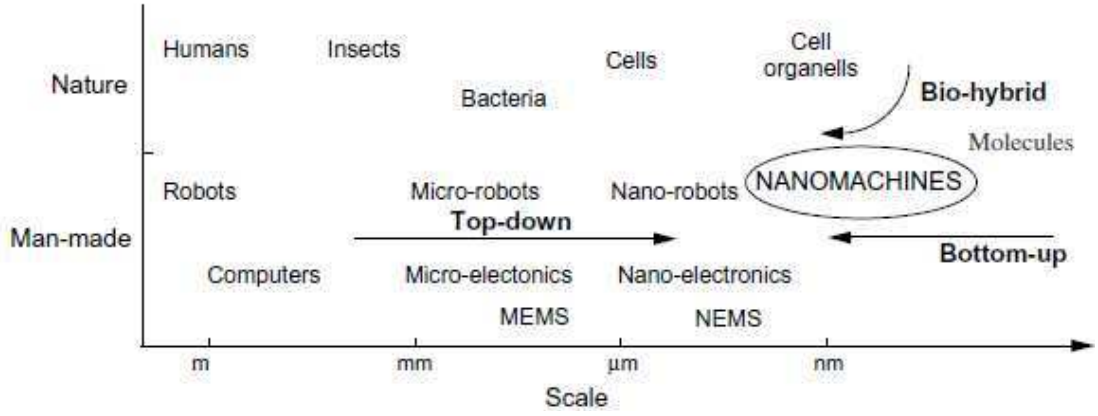

Figure 2.19 : Approaches to nano-machine development [2]

A nano-machine could consist of one or more components, the number of which will determine the complexity of it that can vary from simple molecular machines to nano-robots [17]. Basic nano-machine components are the control, communication, reproduction, and power units. Even though there have been significant improvements in manufacturing techniques, currently it is not possible to build artificially a nano-machine that preserves all of these functionalities. As a result, the research area about nanotechnology is directed towards the biological perspective,

i.e., bio-hybrid, which would enable existing biological creatures to be employed in the nanotechnology applications [2]. Existing biological nano-machines with their optimized architecture and power consumption motivates their usage both as nano-machines and models for the development on artificial creations [2].

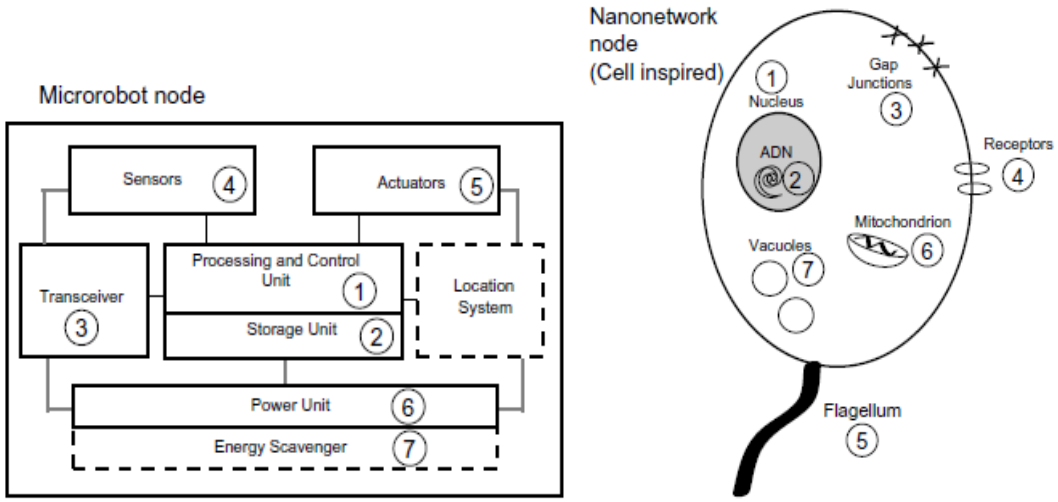

Figure 2.20 : Functional architecture mapping between nano-machines of cell and nano-robot [2]

A cell contains a nucleus as a control unit, uses gap junctions and receptors for communication and is able to reproduce. Cells do have mechanisms for power control, plant cells is also able to generate energy without the need of nutrition with the help of chloroplast. Providing these advantages of the biological perspective, the most appropriate approach to create nano-machines is genetically modifying eukaryotic cells.

2.7.2 Nano-machine Communication Skills

In addition to the benefits of the cells as nano-machines, the communication capabilities are also very important. Communication is the feature that will enable cells to work in a cooperative manner. However traditional communication skills can not be used in nano-scale devices, molecular communication which is the transmission and reception of information encoded in molecules, has its advantages on nano-scale. Unlike other communication skills (i.e. electromagnetic waves, acoustic communication or nanomechanical communication) molecular communication is more realistic due to size and nano-scale framework.

Table 2.1: Comparison of traditional and molecular communication [2]

Communication	Traditional	Molecular
Communication carrier	Electromagnetic waves	Molecules
Signal type	Electronic and optical (Electromagnetic)	Chemical
Propagation speed	Light (3×10^8 m/s)	Extremely low
Medium conditions	Wired: Almost immune Wireless: Affect communication	Affect communication
Noise	Electromagnetic fields and signals	Particles and molecules in medium
Encoded information	Voice, text and video	Phenomena, chemical states or processes
Other features	High energy consumption	Low energy consumption

Nanonetworking with molecular communication increases the capabilities of nano-machines in achieving more complex objectives by cooperation, and in expanding their workspace. Reduced size, biocompatibility and energy consumption are the key benefits derived from molecular communication. Reduced size of the nano-machines and molecular communcation provides a big advantage on applications where the dimension of the involved elements is critical. Biocompatibility is the ability of the nano-machines to operate in biological environments and energy consumption advantage comes from the high efficiency of the chemical reactions [2].

In nanonetworks there exist five different components: the transmitter node, the receiver node, the carrier, the medium and the messages. The communication process including these components starts with the transmitter encoding the message onto molecules. Then transmitter inserts the message into the medium by releasing the molecules to the environment or attaching them to the molecular carriers. The message (either with a carrier or by itself) propagates through the receiver and receiver detects the message. Finally, the receiver decodes the molecular message into the relevant information [2].

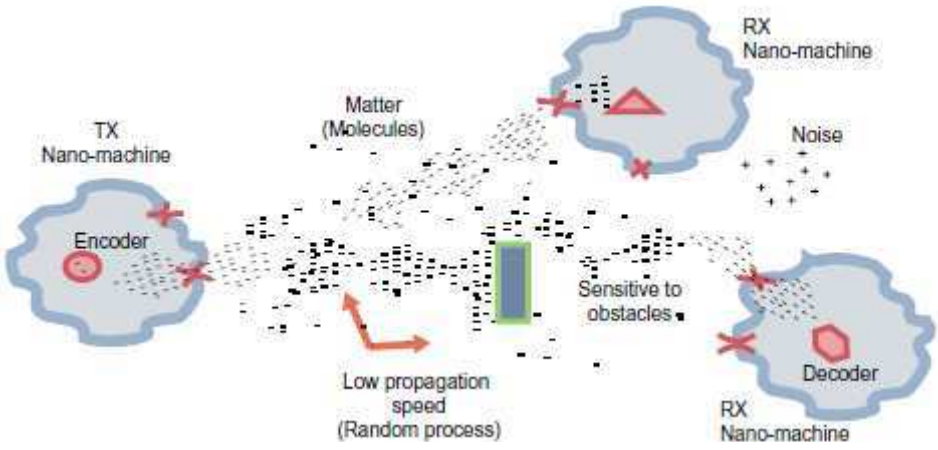

Figure 2.21 : Molecular Transmission [2]

For short range communication among living cells i.e. nano-machines, various mechanism have been proposed. Since most of the intra-cell communications are based on molecular motors, this mechanism is one of the most popular ones. Molecular motors are proteins or protein complexes that convert chemical energy into mechanical work at the nano-scale. They are present on eukaryotic cells in living organisms. Their usage as information carriers is widely proposed. Another short range communication method that can be applied to nanonetworks is calcium signalling. It is one of the most well-known molecular communication techniques. Calcium signalling is used in various tasks in the cells such as contraction, secretion or fertilization. It can be used to exchange information among cells. The big advantage of calcium signalling is that it does not require cells to be physically close to each other. Hence among cells that are separate without any physical contact, calcium signalling does also work. Calcium can propagate in the intracellular liquid by diffusion or using gap junction signal forwarding mechanism [2]. Gap junction is a narrow gap 2-4 nm, between the membranes of two adjacent cells. The most important characteristic of gap junctions is their intercellular channels which tolerates small molecules to pass directly from the inside of one cell to the inside of the other. The gap junction is formed by two hemichannels [18]. The hemichannels should work together in order to form a complete gap junction, which will let molecules go by.

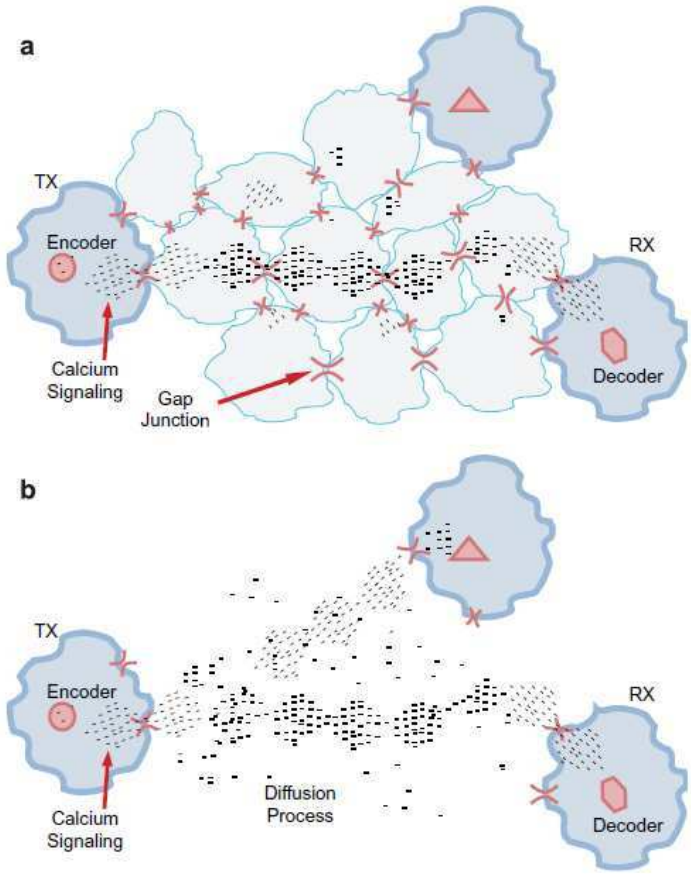

Figure 2.22 : Calcium Signalling Communication System (a) Gap junctions signal forwarding (b) Diffusion [2]

In case of long range communication, distance between sender and receiver ranges from millimetres to kilometres. Long range communication in nanonetworks is inspired from the biological communication systems among ant or bee colonies. Moreover, many of the mammals use pheromones in order to establish the long range communication. Communication mechanism using pheromones is similar to the short range techniques. Pheromones are released from the sender and detected by the receiver. While propagating in the medium, several factors might affect the pheromones which might interrupt the communication. This problem is similar to the noise concept in traditional communication channels. In long range communication, information must be loaded into the molecules. Different encodings in the molecule structure leads to combinations in the information. Receivers in this communication structure are responsible for realizing molecules by molecular receptors [2].

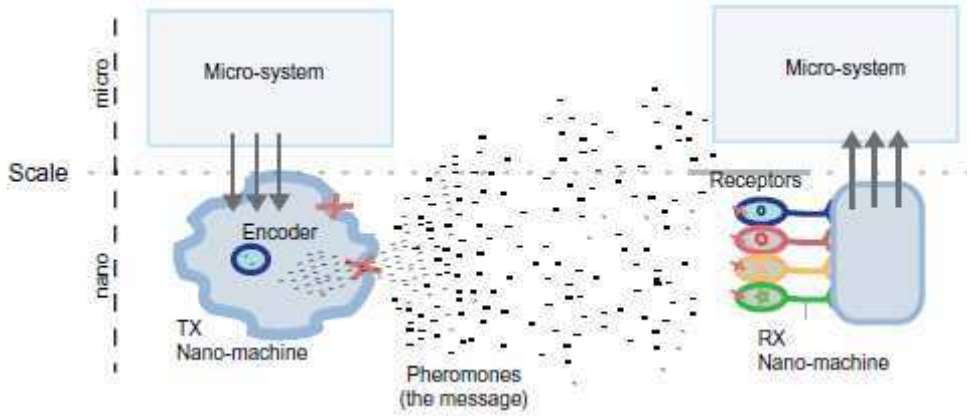

Figure 2.23 : Conceptual diagram of a pheromonal communication [2]

Realizing the pheromones is achieved by the ligand-receptor binding process in the destination. Ligand is the molecule interacting with a specific protein. The interaction in the receptors of the receiver nano-machine enables it to understand the message. Although the implementations of this mechanism involves macro systems i.e. animals, communication mechanism performs in the nano-scale. Long range pheromonal communication includes biological tasks similar to the short range communication. These tasks can be mapped to the existing communication paradigms in the information technology, first of which is the encoding. Long range communication starts in the sender with the encoding process which involves the selection of the specific pheromones to be transmitted. Hence, the information that is desired to be sent is encoded into the appropriate molecules. The second task in the long range communication is transmission, which is the process of releasing information encoded molecules into the medium. Transmission is then followed by the propagation of the pheromones from the sender to the receiver. Propagation is simply the diffusion process in the communication medium. Environmental conditions such as temperature and pressure effects the diffusion of the molecules. After transmission task is completed, reception of the molecules at the receptors of receiver is completed. Receptors are proteins that are sensitive to pheromonal messages that ensure the information molecule-receptor binding. Finally, decoding of the data in the pheromones must be done in order to interpret the transmitted information at the receiver. It is important to note that today's nano-machines are not able to perform these tasks for the communication; however they are expected to be available in the foreseeable future [2].

3. PROPOSED NANONETWORK WITH THE BIOLOGICAL BACKGROUND

3.1 Cells in the Culture Environment

Artificially created nano-scale machines are not available with current technology in order to employ on nanonetworks. Therefore, this study as most of the studies in the literature about nanonetworking, will take already existing biological mechanisms as base, to propose an improved nanonetwork structure. Concerning the cell structure and dimension, it would be very hard to study their behaviour on certain conditions in the living organism. This would also hinder the modifications and effects that might be applied on the cell structure. In order to avoid these drawbacks, cell culture environment, in which cells are observed in vitro (glass) instead of in vivo (life), is preferred in this study. Cell culture permits investigations on the physiology or biochemistry of cells, and testing effects of various compounds or drugs on specific cell types. Animal or plant cells, removed from the tissues, are able to grow in the culture environment if appropriate conditions are supplied. When carried out in a laboratory, this process is called cell culture [19]. Considering the nanonetwork environment proposed in this study, the culture process allows a single cell to act as independent unit like a microorganism. In culture, cells are able to divide and increase in size when nutrient supply is adequate [19]. Cultures might contain cells of same type or diverse set of cells from different types. The culture environment proposed in this study contains same type of plasma cells from the same organism. Hence, all of the cell will have the same DNA sequence in their nucleus forming a homogenous population; however they will also be serving to diversity using the mechanism of antibody production which is possible by using different parts of their DNA database while synthesizing protein. Plasma cells, differently from other types of cells (e.g. epithelium, liver etc.) are not in the affinity of binding together tightly which would paralyze the nanonetworking environment. They can survive in the culture environment without binding together.

3.2 Molecular Communication

After providing their visibility through cell culture environment, the most important step in building the nanonetworking environment is the communication protocol between the nano-elements or cells in this case. In order to enable communication between nano-elements a communication paradigm other than conventional communication skills is needed. Molecular communication which is an already working way of communication between cells is also a suitable area to be inspired, for the upcoming artificially created or biologically modified nano-elements' communication. In short range or long range molecular communications, information is loaded into molecules at the sender, and the information loaded molecules propagate to the receiver using the propagation system in a controlled manner. This communication paradigm needs detailed design challenges on nano-elements (sender/receiver) and the propagation system [4].

The basic requirements of the communication paradigm are the nano-machine identification, transmission and propagation systems. Host identification mechanism in nano-machines is the most crucial step which will affect the remaining design of the communication system. In the existing mechanism of cells the membrane proteins are used to identify the cells in a tissue or organ. However this mechanism does not provide a unique addressing scheme for the cells. That's to say; when cell communication is considered, a unicast messaging is not possible with the membrane protein identifiers. Since most of the cells in a tissue do work in a correlated fashion, they have similar identifiers in their membrane, which yields them to get influenced from the same hormones or other factors in the blood. Membrane proteins might be the correspondent of multicast addressing, since cells that are in the same tissue are affected or not affected from a factor. Though, this mechanism is not sufficient from the information technology's point of view. In nanonetworks, unicast addressing schema is crucial in order to be able to communicate with a unique nano-machine. This brings up the necessity of a new communication protocol between nano-machines or cells. The proposed propagation system in [3, 20] uses DNA hybridization/strand exchange in order to determine the loading/unloading sites of elements. To make this system work each sender and receiver cell is identified with a single-stranded DNA (ssDNA) with the purpose of unique addressing. This new identification style in the cell environment broadens the biological cell identification

methods using membrane proteins, and gives a unique id to each cell in the environment. At last the unique address assigned to each nano-element in the environment provides the required networking background for the unicast messaging.

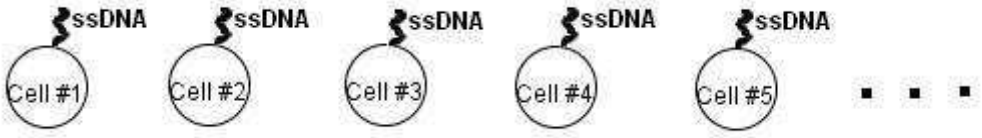

Figure 3.1 : Unique ssDNA attached cells

3.2.1 Propagation and Transmission Systems

3.2.1.1 Ion Spreading Based Short Range Communication

Providing that each cell has a unique address with the help of ssDNA's attached to their membranes, the next issue is the propagation and transmission system which would enable unicast messaging between the cells. The classical molecular communication is based on the ion transmission. For instance, in models like calcium signalling the sender releases calcium ions through the environment and potential receivers are able to receive these messages with the propagating ions. However the detection of these calcium ions should be above some threshold value in order to be understood by the destination cells. Calcium ions density declines when the distance from the source increases. Therefore, cells that are far apart from the sender are not able to receive the message, due to the loss in the density of calcium ions. This is a physical boundary limiting the communication distance of the cells. In these kinds of communication models, neighbours of a nano-machine are determined by the communication radius of it. This area is the limited region around a nano-machine in which it is able send and receive messages. Besides the distance, communication radius is determined by the characteristics of the molecules used as information carriers and the physical conditions affecting these carriers in the environment like temperature, pressure etc. Finally the cells inside the communication radius of a cell are considered as the neighbours. For instance, in the Figure 3.2 : there are three nodes (C2, C3, and C4) inside the communication radius of the nano-machine C1, the nanonetworking algorithm would allow C1 to converse only with these three cells if traditional methods of molecular communication is used.

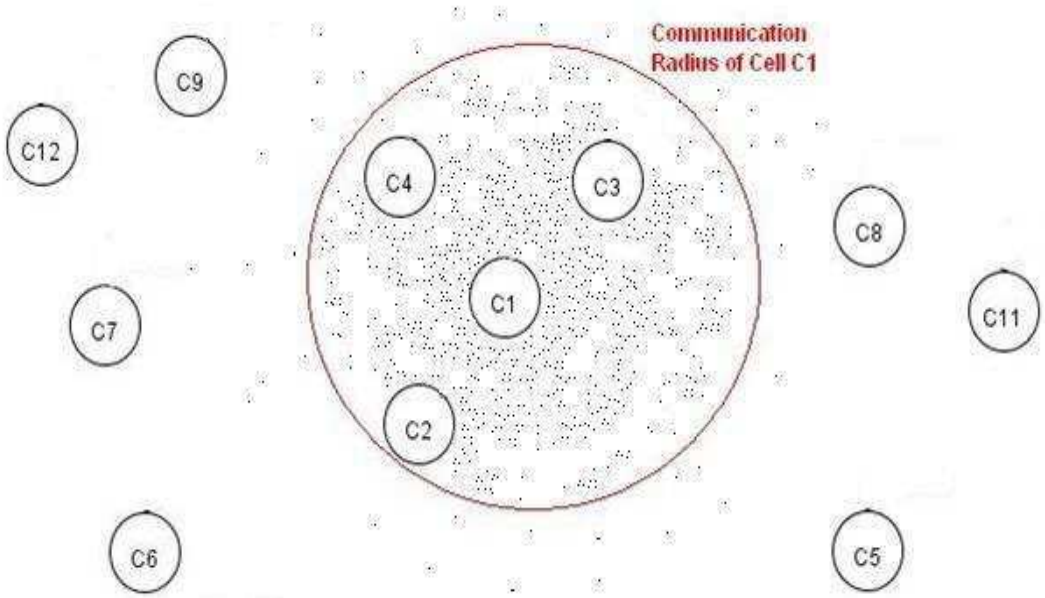

Figure 3.2 : Communication Radius of a Cell with the Ion Communcation scheme

Ion based communication schema brings out other problems in nano-machine designs, such as neighbour detection problem. In this communication method, nano-elements need to know their neighbours and store these neighbours in some kind of database in order to be aware of their communicating bodies. The awareness of neighbours at a time unfortunately does not mean that the nano-element always has an updated database of its fellows. Since the culture environment in which cells are living is an active environment, neighbours are always changing their places, resulting in new neighbours and lost fellows for a nano-element. At last, nano-machines should be able to modify their neighbourhood information in their database. However, this would increase the complexity in cells or nano-machine production.

3.2.1.2 ssDNA Based Short Range Communication

In order to overcome the drawbacks of ion based communication ssDNA based short range communication is proposed [3, 20]. This short range communication mechanism would also benefit from the advantages of ssDNA's attached to the cell membranes which are used for the identification of the cells. To realize this new short range communication, propagation and transmission systems which are also based on ssDNA's are used. Hence with this ssDNA based short range communication s addressing schema nano-machines are able to send/receive messages to/from other nano-machines without the boundaries of communication

radius. In traditional molecular communication schema, a sender generates molecules and encodes information into the generated molecules, then diffuses the information encoded molecules into the transmission environment. The sender might encode the information into the molecules using different molecules for different messages or concentration of the information molecules can be used [4]. In this conventional approach in molecular communication, molecules are directly released to the propagation environment, which generates the communication radius drawback. In order to stay away from this problem, molecular containers that encapsulate the information molecules during the propagation from sender to receiver are used. These containers provide a mechanism to transport different types of information molecules in diverse environments, since it is able to separate the molecules from the environment [4]. This isolation also prevents denaturalization of molecules in the environment, though it has huge benefit on communication distance with the long lasting molecules. In addition to their benefits in communication range molecular containers also provide a basic security mechanism since the communication molecules can not detected by other cells in the environment other than sender and receiver. In the realization of this molecular container mechanism, the most applicable method in terms of biological perspective is vesicles. Given that vesicles are already existing objects, any extra effort to create artificial molecular containers won't be needed.

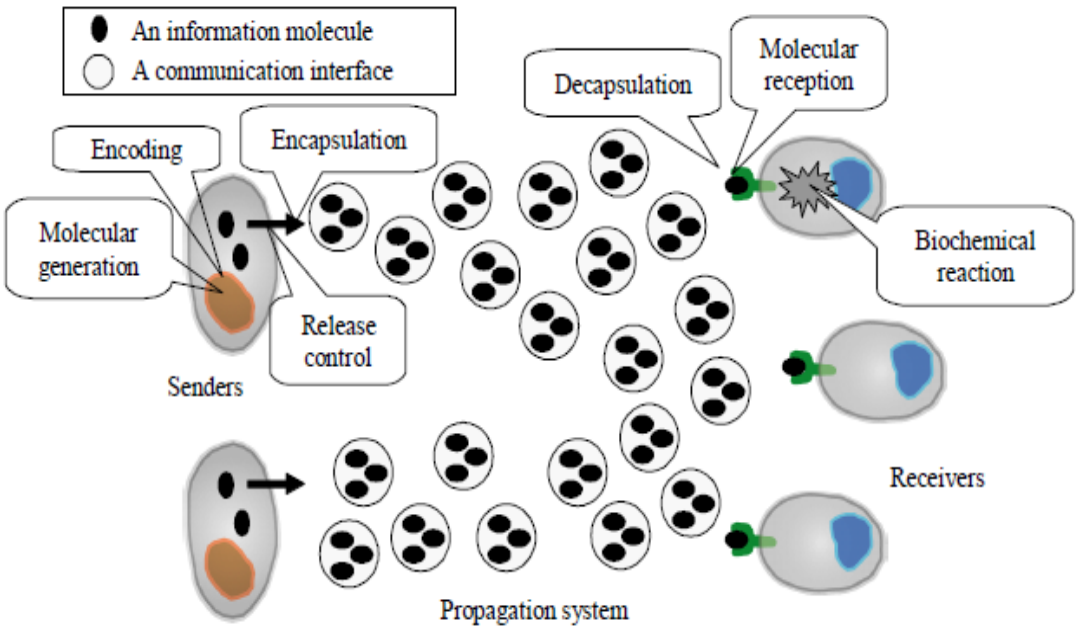

Figure 3.3 : Molecular communication system [4]

The challenge about vesicles is the loading and unloading of information molecules to and from the vesicles. Luckily, in the biological world there is a solution to this problem which is called gap junction. Gap junction is an inter-cellular communication channel formed between two neighbour cells. The proposed mechanism in [4], which is also preferred in this work, uses gap junctions in the implementation of vesicle-based communication interface. The key point in the transmission mechanism is the gap junction hemichannels in sender, receiver and vesicles. It is crucial that all of these elements have half of the junction. Therefore neither vesicles nor the cells (sender/receiver) are able to spread the information molecules into the intracellular liquid. These gap junction hemichannels should come together in order to form a complete channel which is suitable for transmission of information molecules. Two gap junction channels are formed in a message flow, one at the sender (between sender and vesicle), another at the receiver (between receiver and vesicle) side.

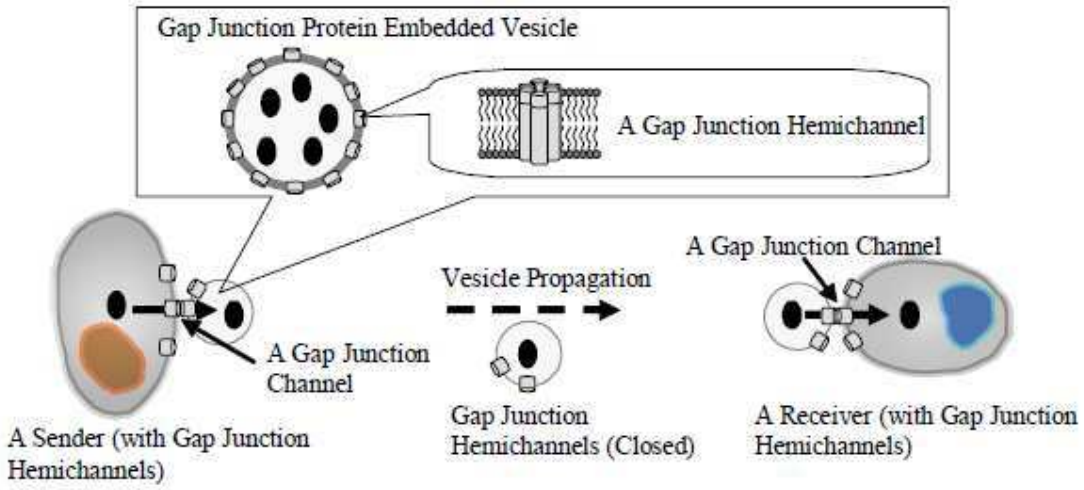

Figure 3.4 : Molecular communication interface using gap junction [4]

In the formed gap junction channel, information molecules are transferred to the vesicle. The movement of the information molecules depends on the difference in concentration. Finally the vesicle disengages from the sender cell, and gap junction channel between the cell and vesicle is closed. Unlocking between the gap junction hemichannels leaves the vesicle into the propogation environment. Vesicle containing the information molecules now needs to propagate through the receiver. In case of molecular communication without vesicles, this propagation should be done as fast as possible, because information molecules (e.g. ions) denaturize in the intercellular liquid after a while. However, with the help of vesicles there is not such

a limitation on the propagation time. Although vesicle based molecular communication has this advantage on time limitations, propagation speed is crucial in terms of communication speed.

In both cases of molecular communication, the speed of the communication is not very fast, since ions or vesicles oscillate in the intercellular liquid, and their movement through the destination is not conscious. With the intention of increasing this communication speed, the first and most important issue is directing the vesicles through the destination. Since external physical intervention to the environment is not a choice here, this work does choose an existing biological mechanism for conscious movement. Eukaryotic cells do have an internal mechanism for transporting molecules along microtubules (cytoskeletal tracks). There are five cargo carrying motors in the early eukaryotes, the number of these motors increases with the evolution [21]. Microtubules are like carriers inside the cell. They are able to move vesicles, granules and even organelles. In addition to transporting with microtubules, eukaryotic cells also have mechanisms called biological motors (e.g. kinesins) for loading/unloading of cargo molecules (e.g. vesicles in this work) [4]. So, biological motors and microtubules work together to manage the transportation inside the cell. This correlated transportation mechanism is preferred in this work for the conscious vesicle propagation between the ssDNA attached cells. Benefiting from the microtubules and biological motors will improve the propagation time of the vesicles, which will also yield to less occupation of vesicles. At last, not only the speed of propagation is increased but also number of vesicles needed in the intracellular liquid in order to transport information molecules also decreases. In order to accomplish collaboration between the ssDNA attached cells and vesicle based propagation system, the use of the ssDNA's also on the vesicles is also needed. So that, vesicles carried by the propagation system using microtubule mobility on kinesins is sent to the correct destination.

To integrate the ssDNA based unique addressing schema of cells with the propagation system described above, vesicles and microtubules should also be identified with ssDNA's. The correlation between these ssDNA's depends on the DNA hybridization among them. In order to improve the probability of matching, length of ssDNA attached to the microtubules is shorter than the vesicles. So that, vesicles can serves as general carrier in the nanonetwork. The length of the ssDNA

attached to the vesicles is at the same length with the sender and receiver cells. This provides the single destination property for a particular vesicle, resulting in unicast messaging. The workflow of the propagation system start after sender fills the information molecules inside the vesicle. For a vesicle filled with information molecules, to be loaded on a microtubule passing through the sender cell, the vesicles ssDNA and microtubules ssDNA should be complementary of each other. If the ssDNA's of vesicle and microtubule are complementary, then the vesicle is selectively loaded into that microtubule using DNA hybridization. Otherwise vesicle would remain at the sender cell, waiting for a microtubule with complementary ssDNA. In the positive case, the vesicle-microtubule complex is transported by the microtubule mobility on kinesins (e.g. biological motors) towards the destination cell. (Figure 3.5 :) When the microtubule-vesicle complex reaches the given destination cell, the ssDNA of the destination cell should be complementary of the vesicle in order to achieve autonomous unloading. When the microtubule is passing by the destination site, the vesicle with the complementary ssDNA to the destination cells ssDNA, is selectively unloaded from the microtubule through DNA strand exchange [4]. In order to achieve the loading/unloading no external effect is needed, which makes the workflow simpler for the design of nanonetworks.

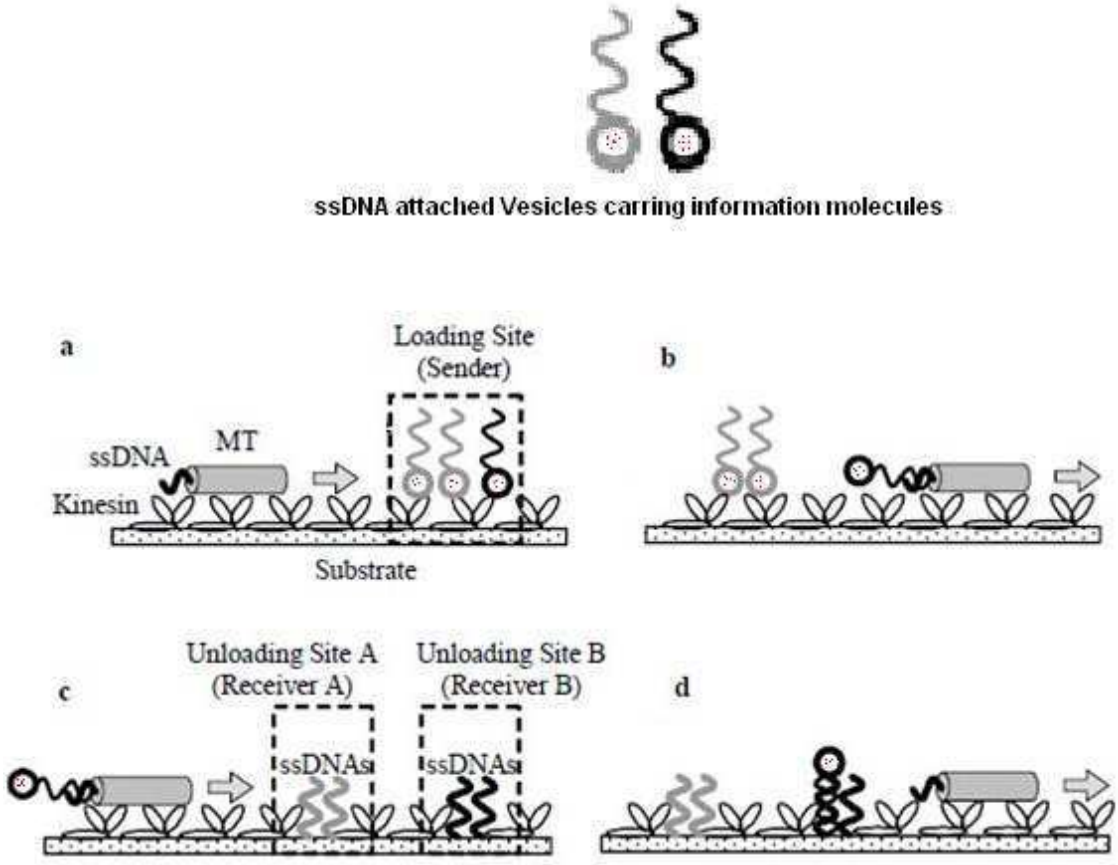

Figure 3.5 : Molecular propagation system [4]

Finally, the ssDNA based short range communication has advantages over ion based communication especially on two topics. The first benefit of this system is its does not impose communication radius boundaries on the nano-elements. Therefore every nano-element is able to communicate even if they are not in the range of molecular denaturalization. The second benefit of this propagation and transmission mechanism is the conscious movement of the information molecules with the help of microtubule mobility on kinesins. This conscious propagation system would decrease the propagation time with less microtubule requirement because it will decrease the microtubule occupancy rate with fast propagation.

3.3 Proposed System for Database Requirement

Transmission and propagation systems using ssDNA requires the cells that are taking part in this communication system to be aware of their neighbours' addresses in order to get in touch with them. If the molecular communication using ions were preferred then each cell would also need to be aware of its neighbours in its

communication radius, because it is only able to communicate with those cells. Since cells are not in a fixed place in the cell culture environment neighbours of a particular cell could change at any time which makes this mechanism harder. Therefore in ion base communication, cells would not only be responsible for their potential neighbours' ssDNA addresses but also they should be keeping track of the changes in their communication radius. This mechanism would also require a modification capability on the database of the cells. Neighbours of each cell might change in a while, which would bring up the necessity of adding new active neighbours in the database and deleting the old ones which are not in the communication radius right now. Luckily, in the proposed communication schema in this work it is not the case. In the communication method used in this work, each cell is able to communicate with any other cell without any boundaries like communication radius, or molecular denaturalization with the help of ssDNA enabled unique addressing and vesicle based propagation system. Therefore, cells do not have to modify the information in their database depending on their active or passive neighbours, providing that all of them are active. Nevertheless, the necessity of a database system for storing other cells addresses (ssDNA's) does still exist. Two methods that can be used as database implementation on cells are proposed in this work. Using DNA or humoral immunity as a database are the techniques that are compared. Due to motivations which are explained below, using homural immunity as a database implementation is prefered in this work.

3.3.1 Using DNA as a Database

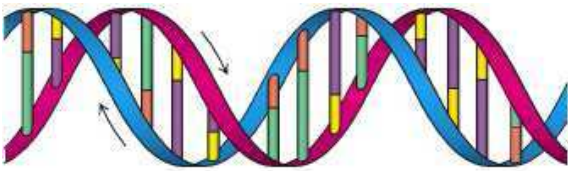

Figure 3.6 : The Double Helix. The double-helical structure of DNA proposed by Watson and Crick [9]

Since there is not a disk space that can be used to store the neighbourhood information in the cells, which are used as nano-machines in this study, another place to store this information is needed. The data that is needed to be stored is ssDNA's of neighbour cells, which makes the DNA sequence of eukaryotic cells, seem to be like an appropriate candidate for database simulation. The potential neighbours in the cell

culture is the same for every nano-machine with the help ssDNA based propagation mechanism, therefore it is meaningful to have the same database on every cell. Fortunately this is the exact case in the cells that are taken from the same organism. Even if cells are taken from various tissues or organs, the DNA sequence of them are same providing the fact that they are obtained from the same living creature. For instance, if these cells are acquired from a human's different tissues like epithelium, muscle, liver, lung or even an eye, they will have the same DNA sequence in their genome. The only difference would be the active parts in the DNA sequence that can be used to synthesize protein. In this work plasma cells from the same animal is taken. Namely, all of the cells in the culture environment will have the same database. Providing that each cell has the same database, now it is crucial to use the already present information in this database while creating the ssDNA's synthetically. Since these ssDNA's are artifically created in laboratory environment, applying the arrangement currently existing on the cell DNA's to the ssDNA's would not be a problem. In this way each cell having the same DNA sequence in their nucleus will be able to have the same set of address space in their database.

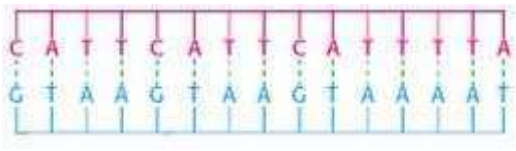

Figure 3.7 : A small section of the sample cell DNA

Assuming that the above sequence is from the sample plasma cells taken from a single organism, then the following ssDNA's should be artificially attached to these cells with the aim of unique identification in the nanonetworking environment. The length of the ssDNA's attached to the cells may vary according to the number of cells in the environment.

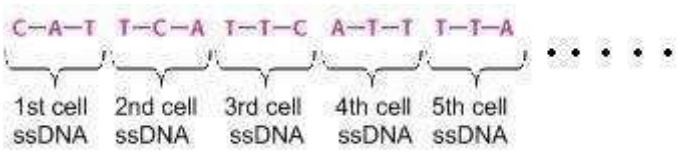

Figure 3.8 : ssDNA's attached to nano-elements (length of ssDNA may vary on different nanonetworking environments)

Besides having these advantages in database simulation on DNA, there are some boundaries brought by this mechanism. First of all, the number of cells in the culture

environment would be limited. Total number of cells could be at most equal to the length of DNA sequence divided by length of ssDNA sequence used for identifying the cells.

$$\text{Total Number of Cells} \leq \frac{L_{\text{DNA SEQUENCE IN CELL}}}{L_{\text{ssDNA SEQUENCE USED FOR CELL IDENTIFICATION}}} \quad \textbf{(3.1)}$$

Second disadvantage of using DNA as a database implementation would be faced when new cells are required to be added into the culture environment or when existing cells divide. In order to add the new cell addresses into the database of the existing cells, ssDNA addresses of these new cells are needed to be activated on the DNA of the existing cells in the culture environment. This problem is caused by the DNA protein synthesis mechanism which determines the active sections in the DNA. Active sections in the DNA are the parts that are available for protein synthesis. Those are the parts that are available for that particular cell to read. If all of this limited section of active DNA region is assigned to ssDNA addresses of the existing neighbours then cells would not have a place to store the addresses for new cells. This requires a modification on the active sections of the cell DNAs. Only with this modification, existing cells would be able to save of their new neighbours' ssDNA addresses. However this modification is not a very easy thing to do and only known way of these kinds of modification is in root cell methodologies. Even in root cell studies it is not possible to add new active sections to that cells DNA but a new cell with those activated parts in DNA is generated from the root cell. Finally, with the drawbacks of limitation on the number of cells in the culture environment and unavailability in adding new cells into the nanonetwork, DNA is not a very suitable candidate for our database requirement.

3.3.2 Using Humoral Immunity as Database

Due to mentioned problems with DNA as database mechanism, another method should be implemented in the nanonetwork. This work proposes the use of humoral immunity as a database implementation in the cells. The mentioned humoral immunity mechanism in the cell culture requires plasma cells to be used. All mammals in their plasma cells are able to synthesize huge amounts of specific antibody against any foreign thread within a matter of days, after being exposed to it. Antibody diversity is maintained by the amino acid sequences in the variable regions

of heavy and light chains. The diversity mechanism of antibodies consists of multiple variable genes (V) separate from a single constant (C) gene in embryonic DNA. V genes are joined to C gene in order to maintain the diversity of antibodies. Besides V and C genes there are J (joining) genes located near the C gene in embryonic cells. In the differentiation of an antibody production, a V gene becomes merged to a J gene in order to complete the variable region of the gene. At last, a single V gene is linked to a J gene to form a VJ region. This V and J genes are selected randomly and the place of the joint between them is also random. Therefore, many VJ combinations are generated by this randomness. Randomly formed VJ region is then joint with the constant gene (C), resulting in a VJC gene sequence. Then from this rearranged gene, mRNA is produced by transcription, which will then be used in translation and processing. After all these steps the result is an L chain protein. This diversity generated by gene rearrangements yields to 10^8 distinct antibodies [9]. Recombination of these VJ genes strengthens the diversity between these genes. The crucial point here is that this diversity going up to 10^8 antibodies is supplied by only 40000 genes.

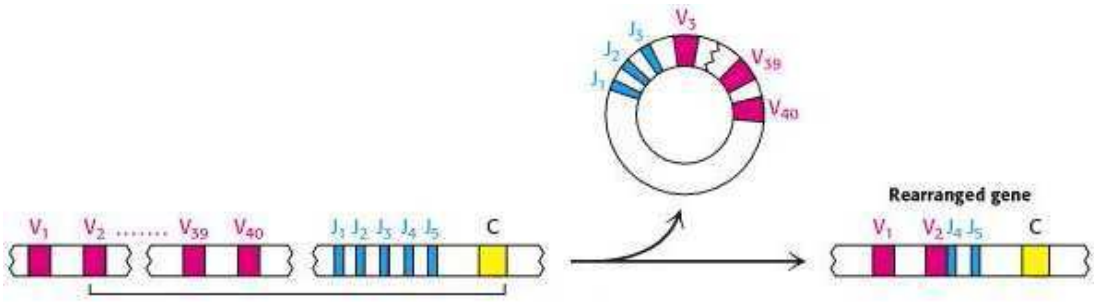

Figure 3.9 : VJ Genes Recombination [9]

After producing specific antibodies for specific antigens, plasma cells are able to remember these sequences which are the actual cause of the immunity. When the body is exposed to same type of antigen again, there is no need to work with gene formations again. That is actually what immunity is called. Their ability to remember previous antigens and the diversity mechanism with the help of VJC gene formations make the plasma cells most suitable candidate for database simulation on the cells in biological environment. This work proposes the practice of the this mechanism in plasma cells to be used in the nanonetworking framework. Since plasma cells are able to synthesize various different proteins according to the antigens that they encounter, cells in the nanonetworking environment can also be able to synthesize

proteins according to the ssDNA's of their neighbours. Besides synthesizing proteins for the ssDNA's of their neighbours plasma cells are also able to remember these sequences as if a plasma cell is remembering an antigen's DNA sequence. Previously using DNA as a database was omitted in this work due to some drawbacks. If these issues are checked on the plasma cells and their immunity mechanism it can be seen that, plasma cells does not carry any of these drawbacks. The first issue is the necessity of modifications on DNA in order to add/delete new neighbours. Memory on the plasma cells does not require any of these modifications on the DNA. Therefore it is much applicable method from biological perspective. While not requiring any changes on DNA sequence, plasma cells memory does not have any drawbacks on the data storage capability. They are able to store huge numbers of antigen information for years depending on the type of antigen. Consequently, the ssDNA addresses of the neighbour cells can also be stored for long periods. The second issue was the limitation on the number of cells in the culture environment. Using DNA as a database method has limited the number of cells with an upper bound which is length of DNA divided by length of ssDNA. However in the plasma cells the number in the database of each cell could go up to 10^8 neighbours. This advantage of plasma cells comes from the diversity generated by the VJC gene recombination. The third problem of the using DNA as database method was its disability to accept new cells in the environment. This issue is also solved in the plasma cells. New cell, either coming from a division of an existing cell or a brand new cell added explicitly to the environment would not face this problem. The cell coming from the division would already know all of the neighbours already, and the new cell would learn it as the other plasma cells did. Using plasma cells in the nanonetworking does only bring some restrictions about artificially created ssDNAs on the cell membrane. Since plasma cells should be able to determine and save the ssDNA addresses of their neighbours, these ssDNAs should be compatible with the plasma cell's range of diversity. Namely, the arrangement in the ssDNA should be suitable to be detected and copied with the VJC gene combinations in the plasma cell.

In the nanonetworking schema proposed in this work with plasma cells, mechanism of creating a new antibody for a new antigen is used. A plasma cell does manufacture a specific antibody for that particular antigen in order to protect the body and itself

using the diversity method depending on variable genes. Production of this antibody means that this antigen is added into the plasma cell's database and if it faces the same antigen again after sometime this antibody would be used without the necessity of trying to figure out a new antibody. Immunity with its features described above is used in this work as a database mechanism. In the nanonetworking mechanism, each plasma cell with a ssDNA attached to their membrane is used in the culture environment. At the first stage, each plasma cell does broadcast its ssDNA address to the environment. Other plasma cells receiving the ssDNA information of the broadcaster plasma cell do work in the same fashion like a plasma cell which has encountered an antigen and trying to figure out an antibody for it. As a result the ssDNA address information of the neighbour cell is saved into plasma cell's database exactly like saving antibody information. Therefore every other cell in the culture is able to learn that particular cell's address. This address broadcast mechanism is also taking advantage of thepropagation system using vesicles, so every cell in the culture is able to learn that particular cell's address without the risk of denaturalization or communication radius issues. The final stage of this broadcast mechanism is the cells which are aware of every other cell and their ssDNA address in the environment. This database schema depending on the humoral immunity would not fail under normal lifecycle of an animal cell. That's to say, nanonetworking environment and database schema would not suffer from cell deaths or cell divisions.

3.4 Culture Environment as Habitat

3.4.1 Messaging Between Cells for the Proposed System

On the previous sections of this work, nanonetworking environment on the cell culture environment, ssDNA enabled addressing schema, propagation and transmission systems and finally the ability of the plasma cells to learn the ssDNA addresses of their neighbours are proposed. Using this nanonetworking background, this work does propose unicast, multicast and broadcast addressing techniques. This addressing method would enable division of labour between the cells. In order to realize this addressing schema the ssDNA addresses of the plasma cell will be used. These addresses are synthetically added over those cells as stated previously. Therefore modification on the ssDNA addresses of the cells is the easiest one that can be done without interrupting the biological environment. The only limitation on

ssDNA modification is the plasma cells capability of creating those ssDNA sequences. That's to say, all of the ssDNA addresses that are assigned to the cells, should be producible by the plasma cells in their VJC gene diversity because these addresses are saved into the plasma cells database with this technique. Preserving this condition, ssDNA's can be created and attached to the cells in every sequence. Using this flexibility, this work proposes unicast, multicast and broadcast messaging techniques among cells. This messaging framework depends on the different lengths of ssDNAs that are used on cells, vesicles and microtubules. The length of ssDNA addresses of the cells is assumed to be "n". The proposed propagation system in this work does employ ssDNAs of same length "n" on sender, receiver and the vesicle. Only the microtubules used for carrying vesicles will have shorter ssDNAs.

3.4.1.1 Unicast Messaging Between Cells for the Proposed System

If the sender cell is in the need of sending a unicast message to a specific destination, it fills out the address of the destination cell on the vesicle using the information available in its database. When the microtubule-vesicle complex reaches the given destination cell, the ssDNA of the destination cell would be complementary of the vesicle in order to achieve autonomous unloading. Therefore filling in a ssDNA address of length "n" results in a unicast message to the destination cell.

3.4.1.2 Multicast Messaging Between Cells for the Proposed System

Besides this unicast messaging, it is also possible to send multicast messages. Multicasting does also depend on the complementary relationship between the vesicles and microtubules in the propagation system. This is achieved via smaller ssDNAs in the microtubules. The length of the microtubules is "k" and it is definitely smaller then "n". This smallness helps multiple microtubules to be complementary to that vesicle. Similar to this mechanism vesicle with a ssDNA of length "m" can be used to be complementary to more than one destination cell.

3.4.1.3 Broadcast Messaging Between Cells for the Proposed System

Finally, for the broadcast messaging same logic also applies. In order to make the vesicle complementary to all of the cells in the environment, its ssDNA length is determined as "b". With the help of this small ssDNA section this vesicle's ssDNA address would be complementary to all of the cells in the cell culture.

At last, "n" is the length of the full ssDNA defining a unique destination cell, "m" is the length of the ssDNA that can be used for multicast messaging. "b" is the ssDNA

length suitable for broadcast and finally “k” is the length of the ssDNA used on microtubules. The following relationship between these lengths should be preserved in order to have a operational messaging framework.

$$n > m > b \geq k \quad \textbf{(3.2)}$$

This relationship means that the first “b” base of the ssDNAs must be the same in all of the cells. That is how it can be used as a broadcast address. The suggested addressing schema in this work, enabling unicast, multicast and broadcast messaging, supplies an infrastructure of division of labour between the cells. By courtesy of this addressing mechanism, all of the cells in the nanonetwork is not forced to do the same job. Groups of cell might be employed on different jobs, or same job might be divided into groups of cell in order to increase the speed of it.

3.4.2 Cell Lifecycle

The final issue that needs to be considered in this nanonetworking schema is the cell life cycle. The system should be protected to changes that might occur in the normal operational flow of a cell. The first of the changes that the nanonetwork will face is the cell divisions. In the culture environment, plasma cells would not suffer from hunger, therefore there would not be a case preventing them to switch to G1 phase [11]. After the division of a particular cell, the new plasma cells would be aware of the neighbours just like the awareness of the divided plasma cells in the body to the diseases which are encountered before its division. This awareness hinders the need of another broadcast message flow from other cells to tell their ssDNA addresses to this new cell. The only requirement that needs to be done after a division is the broadcasting of the address of these new cells to the cell culture. So that other cells in the environment would be aware of these new cells in the culture environment. For sure, this broadcast message of the new cells would be after their ssDNA is attached to themselves. The second change that cell culture might face is the cell deaths. Although in the culture environment the cells do not suffer from food shortage, their lifecycle does end after a certain period of time. Death of a cell does not effect other cells in the culture environment, however there might be other cells which are trying to send messages to this death cell. Therefore, the ssDNA address of this death cell should be deleted from the database of other plasma cells. When the antigen awareness of the plasma cells is considered, this mechanism is already present in the

immune system. Such that, when the body does not face with an antigen for certain period of time, then immune system does fail to remember how it defeated that antigen. That is the actual reason of renewing vaccines after a time for humans. Therefore, existing cells would also forget the existence of the death cell after a period due to not receiving its broadcast messages. Living cells do prevent this by sending their ssDNA addresses via broadcast messages in certain periods. The last case that needs to be considered is the infections affecting the workflow of the cells. Nanonetwork might be adversely affected due to the cells with an infection. These cells must be removed from the environment in order to preserve normal workflow of the nanonetwork. In order to achieve the removal of the infected nodes without external intervention, existing biological systems usage is proposed in this work. Since cell structure is taken as a reference in this study, immune system will be the baseline for determining and removing defective nodes. In immune system antibodies and T-cell receptors are used to detect foreign organism and initiating the destruction of the invader [9]. T-cells detect infected cells through their cell surface proteins. For instance, when an activated T cell contacts a virus infected cell, contact signalling between CD95L (cell surface protein produced by T cells) and CD95 on the infected cell will occur. Signal transduction caused by this contract gives rise to programmed cell death in the virus infected cell. This prevents replication of the virus [22]. This method of identification and disposal is proposed for the cell culture environment in this study. At the end, defective nano-elements are removed from the culture environment in order to preserve the communication flow between the remaining non-defective elements. This removal has been achieved with a mechanism similar to the identification method of infected cells in immune system of animals using the membrane proteins. At last, infected cells has been identified and removed from the nanonetwork.

4. CONCLUSION

The major purpose of this research was to propose an improved nanonetworking environment which gives a closer framework to the information technology substructure. The current nanonetworking environments in the literature do employ nano-elements as a chunk of sensors or computation devices. Therefore, nano-elements do not have a unique address which can be used in order to communicate directly. This work has used the ssDNA attaching technique on the nano-elements or cells which overcomes the unique identification problem. The second parameter in the nanonetworking infrastructure that needs to be analyzed is the communication protocol between these uniquely identified cells. For this propagation and transmission system requirement, again the ssDNAs are employed. ssDNA attached vesicles and microtubules ensure the message delivery regardless of the environment conditions. The short range molecular communication techniques in the literature does propose ion based communication which has denaturalization and communication radius problems, because of communication molecules not being protected by a vesicle.

Orientation of the information molecules through the destination is also proposed in this work, solving the free oscillation of the molecules in the intracellular liquid. Providing the unique addressing and consistent messaging framework in the nanonetwork, the requirement for a database system in the nano-elements has given rise in order to store ssDNA addresses of neighbours. This work proposed a database mechanism using humoral immunity of the mammal cells. With the help of the proposed database each nano-element has its neighbours ssDNA addresses in hand. Hence, it would also be possible between nano-machines to have a complex messaging structure which is not possible in biological capabilities of the cells. Unicast, multicast and broadcast messaging between cells is also possible employing the proposed substructure. This messaging framework will broaden the possible applications on nano-machines. Another advantage of the proposed database

mechanism and messaging structure is that their workflow is not influenced within the cells lifecycle. That's to say; in the cell culture environment existing cell might die or divide in their lifetime. In case of a death, the remaining cells are able to delete this cell from their database. Upon a division of a cell, other cells are also able to add this new cell to their database. Therefore, the mechanism proposed in this work for messaging and database implementations are not influenced by the normal cell lifecycle events. Only intervention is needed in case of a cell division, since a new cell would need a ssDNA to identify itself in the nanonetwork. Unique addressing, consistent propagation and transmission systems, and database implementations proposed in this work is much closer to the information technology point of view. Currently, the author is working on the applications that could employ the proposed structure.

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