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RESEARCH ARTICLE

A NOVEL APPROACH FOR BIOTECH: ORGAN-ON-CHIP.

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Abstract

Remarkable efforts have been dedicated on the studies of cultured cells over the last few years, which have given us a new insight into the biological control and provided us considerable knowledge about the human pathophysiology. With the immerging technology of microfluidic Lab-on-a-chip we have been able to analyse intercellular communications and tissue-tissue interactions in a more convenient and comprehensive manner. The microfluidic Lab-on-a-chip is undoubtedly the most optimistic platform to address the inherent complicacy of cellular system with 4D analysis and huge experimental parallelization on a single cell level. In this paper, we have summarized a few recent examples of studies that provide a glimpse of what is now possible using human organs-on-chips, and the unique advantages this approach can provide beyond those offered by existing culture systems used to study intercellular communication in vitro.

Introduction:-

As the studies of cells under captivity has led us to new perceptions into biological environment, substantial understanding of human body pathophysiology needs the advancement of experimental systems that allow analysis of interaction between the tissues and intercellular communication in a more Broadway.

Thus, organs-on-chips brings a new powerful approach to confront such problems. Till date our technology has not been so advanced to create a fully functional, working replica of an organ with the right 3 dimensional spatial relationship and accurate construction of the organ down to the cellular level. So, to visualize the activities at the molecular level in real time we have to “reverse engineer” the whole living organs.

Every organ in our human body has an interface between a vascular tissue and a parenchymal tissue that provides the organ with important nutrients, oxygen and delivers immune cells. By the by all the organs also have to experience mechanical forces and as neuroelectrical cues that are very important for normal organ physiology. Thus, if we reverse engineer an organ, it would be critical to reconstruct interfaces between the tissues, ensure vascular perfusion and provide all environment relevant to an organ. These are the minimal design principles that has led to

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the creation of "organ on chips", which now allows in vitro analysis of how cells work within a human tissue and organs to perform basic physiology.

Organ On Chips:-
An organ-on-Chip is a 3-dimentional microfluidic cell cultured device that simulates the workings, mechanics and physiological response of an entire organ in real time. The chip contains hollow micro channels lines that is made by computer microchip manufacturing techniques and these channel are filled with living cell and tissues that are cultured within an organ. Organ-on-chips vary in design and approach between different researchers. Organs that have been simulated by these device include the lung, kidney, bone, intestine and many more.

Lungs-on-a-chip:-
The research using a lungs-on-a-chip that reconstructs the alveoli-capillary interface shows that the insertion of tumor necrosis factor-alpha (TNF-a), airborne pollutants, living bacteria to the space not only induces expression of Intercellular Adhesion Molecule 1 in the underlying endothelium, it also results in enhanced recruitment of circulating human neutrophils when they are perfused through the vascular channel (Dongeun Huh et al, 2010).The human lung inflammation can be mediated using the lung-on-a-chip as they give high visual clarity which results in high resolution microscopic analysis of the cellular interaction of the lungs. Also, by introducing cyclic suction to micro chambers that run parallel to the culture channel, it is possible to imitate normal breathing motions in the chip by applying a pattern of rhythm of stretching and relaxing.

As the potential to study physiological response at the organ level also resulted in the founding of the transcytosis of the nanoparticles across both the endothelial and epithelial tissue layers, and their absorption into the vascular channel are closely responsive to cyclic mechanical cues. The unknown regulatory process that involves interactions between the immune, epithelial and endothelial human tissues has been uncovered by the use of lung-on-chip as well as their sensitivity to physiological breathing motions. As the organ-on-chip is synthetic in nature it is important to record that it can sometimes lead to an unpredicted findings associated to physiological control (DongeunHuhetal, 2012).

Another form of interaction between the tissues was observed in a human small airways-on a-chip model(Benam, K.H. et al, 2016). When human pulmonary micro vascular endothelium and bronchiolar epithelium are cultured on side to side of a common permeable membrane and put on-chip to mimic Polyinosinic:polycytidylic acid, a proinflammatory response is influenced that is very alike to an acute severe asthma exacerbation as seen in humans, including large increases in the basal secretion of RANTES and Interleukin 6.

Furthermore, this organ level effect looks synergistic, and it is particular in that removal of the endothelium has no effect on secretion of other inflammatory mediators. At the same time, thePolyinosinic:polycytidylic acid stimulated upregulation of E-selectin and VCAM-1 expression in the underlying endothelium stimulates adhesion and rolling of human neutrophils circulating through the vascular channel under flow. Again, because neutrophil adhesion and rolling require fluid shear stress, this response cannot be easilystudied using static transwell co-cultures.Therefore, use of the airways-on-a-chip discloses crosstalk between human lung endothelium and epithelium during inflammation and gives us the conformation about the past studies carried on animal that suggested that during virus induced inflammation the pulmonary endothelium works as the key regulator of the cytokine storm.

Intestine-on-a-chip:-
Studies with human gut-on-a-chipand reconstructing the structure and functioning of the small intestine have allowed to investigate on more complicated multicellular interactions involving human intestinal epithelial cells and immune cells, in the presence of living pathogenic bacteria and commensal microbes. Also being bare to physiological peristalsis-like motions and fluid flow (Hyun Jung Kimet al, 2016).While most of the past studies on gut microbiota have been highly depended upon gene sequencing to understand the microbial strains in the intestine, the gut-on-a-chip has made it convenient to co-culture human intestinal epithelialcell with a living microbiome in vetro(Kim, H.J. et al, 2012;Hyun Jung Kimet al, 2016).Use of this human gut-on-a-chip, which permits one to study multiple potentialcontributing factors alone or in combination, reveals how intestinal cells, immunecells, normal microbiome and pathogenic bacteria contribute to disease development,associated inflammation and host tolerance to infection. In contrast, when human immune cells are flowed through the vascular channel at the same time that the epithelium is exposed to LPS, a significant intestinal injury response is observed that is reminiscent of thatseen

in patients with inflammatory bowel disease. Because it is possible toanalyze the effluent of the epithelial and vascular microchannels independently,this approach also led to the identification of a specific combination of four cytokines that are secreted by the epithelium in a polarizedfashion into the vascular channel in response to stimulation with LPS, butonly when immune cells were present. This cytokine combination is necessaryand sufficient to induce the intestinal damage produced by the combination ofLPS and immune cells in this model.

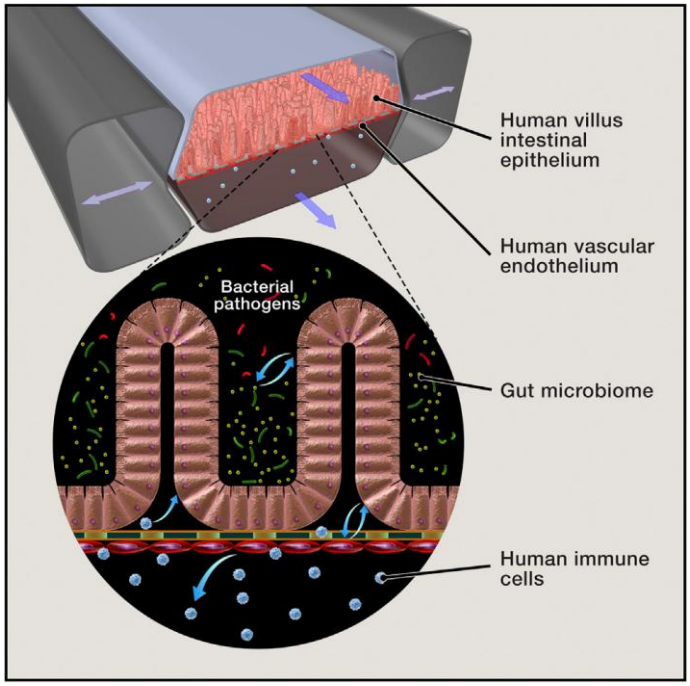

Fig 1:- The above figure displays the cross-section of the human intestine-on-a-chip with a higher magnification showing two parallel channels consisting of a flexible optically clear polymer divide by a porous extracellular matrix made of the same material. It also consist of a hollow channel on both the sides by which suction is applied to produce a cyclic motion of stretching and releasing of the central membrane to mimic peristals in motion. On the top of the surface of the membrane the human villus intestinal epithelium is cultured and expose to a fluid flow in the luminal channel, while on the opposite surface of the membrane human vascular endothelium (red) is grown where it is exposed to an uninterrupted flow of culture medium(Dongeun Huh et al, 2010).

Kidney-on-a-Chip:-

There are more than 10 types of kidney renal cells, these cell are highly organized in a 3-dimensional network encircled by complex vasculature and extracellular matrix (Figure 1 A,B) (Tiong, H.Y. etal, 2014).The xenobiotics excreted by the renal is done by a very complex process of filtration by glomeruli and secretion, reabsorbstion by the tubular apparatus. The renal tubular system is the key element for the development in drug interactions including cellular efflux,influx and cellular metabolism. (Figure 1C)(Tiong, H.Y. etal, 2014).The influx take place at the apical membrane of renal proximity tubule epithelial cells, and the members of the solute carrier family mediate with it. At the apical membrane the efflux is mediated using ATP-bindings cassette transporters. Also, the detoxification is done using efflux facility through multidrug and toxin extrusion transporters (Nigam, S.K.,2015).Liver is more prominent than the renal drug metabolism, but is physiologically relevant and includes cytochrome P450 and Phase-II enzymes (Knights, K.M. et al. 2013; Gundert-Remy, et al,2014).

C.1.Technical and Biological Challenges for a Kidney-on-a-Chip:-

The technical challenges that are mainly faced by kidney-on-a-chip is reliability and usability. Whereas, the biological challenges that it faces can be placed in the context of IVIVE. The improved system must be more robust, reliable and reproducible. The most important parameter that is to be noted is that to gain an acceptance in drug efficacy and toxicity studies is that the biological properties of the chip must be constant (Regehr, K.J. et al,2009;Toepke, M.W. and Beebe, D.J., 2006).

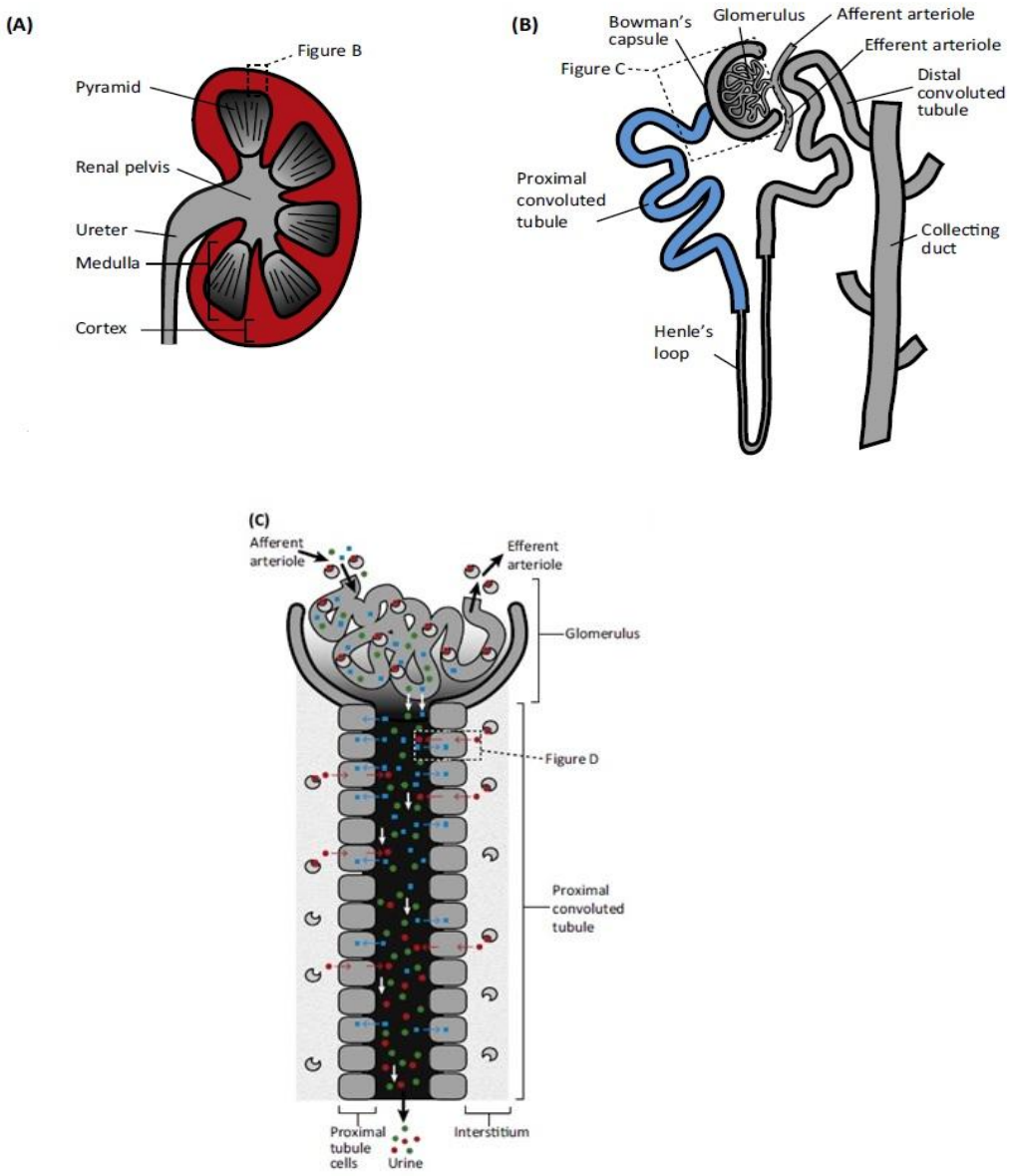

Fig 2:- Renal Transport and Solute Handling. (A) The macroscopic division between medulla and cortex is demonstrated using sagittal section of the kidney. (B) The Bowman's capsule is connected with the proximal convoluted tubule. The tubular structure can be anatomically and functionally subdivided into sections that extend into the medulla. (C) Glomerulus depicted in green and blue filters the solute in the serum, whereas protein-bound metabolites depicted in red are secreted via transporter proteins that are expressed in the proximal tubule epithelium.

Placenta-on-a-chip:-

Placenta play a very crucial role in the process of reproduction. Its most vital task is the interchange of exogenous and endogenous substance, which allows adequate supply of nutrients, excretion of fetul metabolic waste , and protection against potentially harmful agents, such as bacteria, xenobiotics , parasites and viruses(JiSoo Lee et al, 2015).

The mechanisms accountable for these critical process are: (1) facilitated diffusion; (2) Passive diffusion; (3) endocytosis/exocytosis and (4) active transport (Knipp GT et al, 1999).Furthermore, the dynamics of placental transfer are influenced by physical and biochemical factors, including umbilical blood flow, placental metabolism, utero-placental and transporter expression/activity in the placenta. Studies on placental transport have been using ex vivo placental perfusion systems, vitro cell cultures and vivo animal models.

However, they largely fail to reconstitute the physiological structure and microenvironment that profoundly influencetransport processes, raising questions regarding their adequacy as an experimental platform in the study of this placental function. In some cases, humans are directly used to study the placental transfer using therapeutic agents such as hormones and antibiotics. Nevertheless, such studies have a lot of difficulties as they are time consuming and the risk of exposure of the fetus (Prouillac C and Lecoeur S. 2010;Giaginis C et al, 2011).

All these disadvantages are overcomed when the studies are done on the Placenta-on-a-chip microdevice that provides compartmentalized perfusion co-culture of human trophoblasts (JEG-3) and human umbilical vein endothelial cells (HUVECs) on a thin extracellular matrix (ECM) membrane to create a physiological placental barrier in vitro. With the increasing availability of human tissue for laboratory studies, alternatives to animal models have been developed that rely on intact human placental tissue (Myllynen P et al, 2013). Although these new types of model systems provide advantages in recapitulating human-relevant physiology, the lack of standardization in this strategy often leads to conflicting results due to lab-to-lab variability (Myllynen P. et al, 2013). Similarly, other ex vivo approaches, such as placental perfusion models (Giaginis C et al, 2011) have limited ability to dissect the process of placental transfer and to reveal mechanistic insights into its biological underpinnings at physiologically-relevant length scales. Cell culture systems have been successfully used in improving the understanding of placental transfer and metabolism (Liu F. et al, 1997).

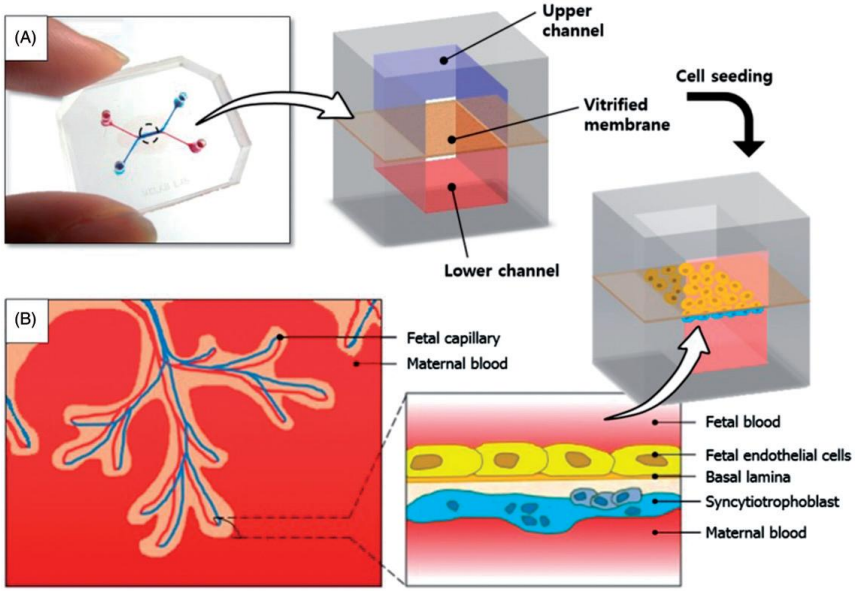

Fig 3:- A placenta-on-a-chip microdevice: (A) Placenta-on-a-chip is composed of the upper (blue) and lower (red) Polydimethylsiloxane (PDMS) chambers separated by a collagen membrane. (B) Trophoblasts and endothelial cells are co-cultures in close proximity on the opposite sides of the membrane that forms a placental barrier(JiSoo Lee et al, 2015).

Cancer microenvironment on chip:-

Broadening our knowledge towards the mechanism of malignancy and resistance to therapy is important for developing a promising design of an anticancer drugs. The immerging technology of microfluidic Lab-on-a-chip is undoubtedly the most optimistic platform to address the inherent complicacy of cellular system with 4D analysis and huge experimental parallelization on a single cell level.

A large diversity of DNA and protein based microarray technologies have been immerged facilitating fast growth in the field of clinical oncology. The shift of traditional methods to a microfabricated format gives a view to enlarge both the sampling throughput and the resolution of analysis, mainly in technologies involving microfabricated format gives a static readout of studied variables, and as they are system specialized in only taking a snapshot of the intermittent cellular reactions (Wlodkowic D. etal, 2010).

Furthermore, the capability to analyse living cells in real time is lacked by the technologies that are based onmicrofabricated array format. The basic nature of the tumor cells is based on the molecular signalling events, giving the ability to adapt to microenvironmental changes (Skommer J. et al, 2010). Significantly, numerous switches, fail-safe mechanism and variable response kinetics succeeding anticancer drug treatment, are malignant cells.

Moreover, chemical and physical cell to cell communication influences the dynamic and heterogenic evolving molecular ecosystem where cancer cells react to therapy (Skommer J. et al, 2010). With such complications with the various cell types and variables acting at the same time, needs dynamic study of many variables at the same time (Ta´ rnok A et al, 2006). As stated, microfluidic based technologies are the most promising route to address the complexity of the cellular system (Whitesides GM, 2006).

Microfluidics is highly focused on manipulating liquids in networks of microchannel with very small dimension between 1 and 1000 mm. At such micro level fluids show different physic-chemical properties, when compared to their behavior at macro level (Squires TM,2005).Due to local diffusion rates the fluid flow in microchannels is laminar thus giving a technique where convective contributions can be negated and the supply of drugs, gases and nutrients to cells can be monitored and understood (Squires TM,2005).

Most importantly, the dimensions of cells and blood vessels are comparable with the dimensions of microfluidic environment. Therefore, drug and gas diffusion rates, stress, shear and even cellular niches can be artificially recreated on the chip. These characteristics make microfluidic technologies the best solution for the emulation ofartificial tumor environment (Walsh CL et al, 2009).

Tumor microenvironment is a very important component for cancer biology, containing response-predictive information and abundant targeting opportunities (Joyce JA, Pollard JW,2009). The tumor microenvironment is a very crucial component as it contains specific combination of cell types and soluble factors that result in a major event relevant in survival, proliferation and hematogenous spread of the malignant cells.As the complexity of these microenvironmental condition are very high it is very difficult to reconstruct using conventional methods (Joyce JA, Pollard JW,2009). Thus, current method to reconstruct a tumor microenvironmet are very challenging and do not provide flexible analysis of cancer cells.Whereas, microfluids platform can amend many of these limitaions and reproduce physiological microenvironment that is normally encounted by human body cells.

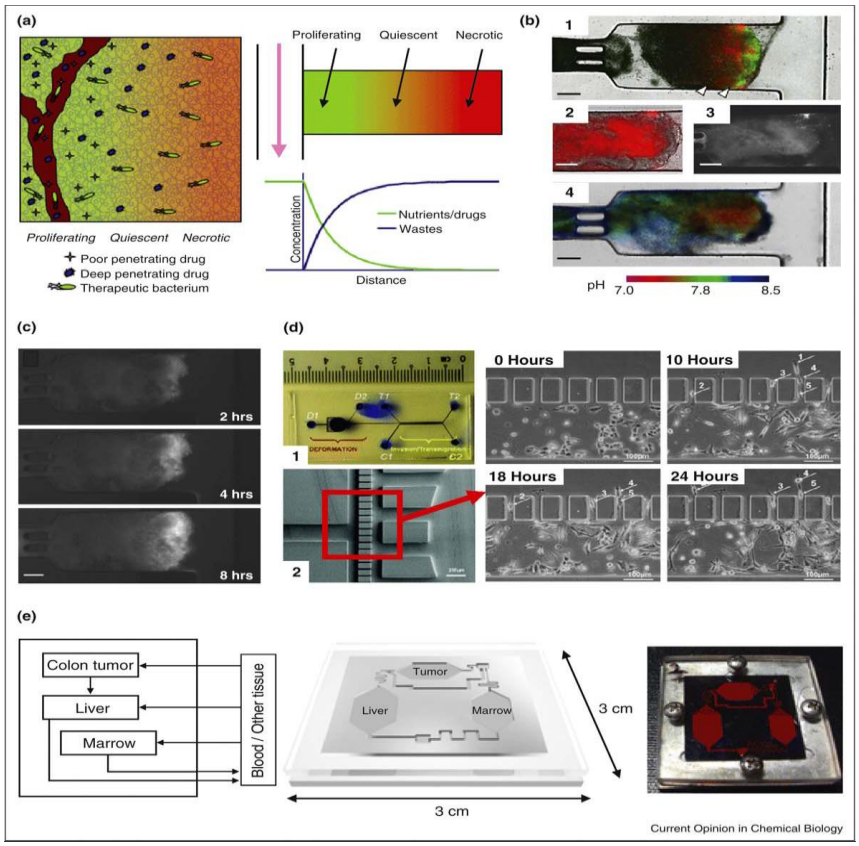

Fig 4:- The above figure are the studies of cancer microenviorment on lab-on-a-chip devices.
(A) The microenviorment in the devices reproduce the surroundings of blood vessels in a tumor. Creating various zones of quiescent (transition), proliferating (green) and necrotic (red) tumor tissue that affect anti-cancer drug penetration and efficiency.(B) The zones of proliferating, viable(green) and dead cells(red). Cellular pH gradient is also evident with acidic and alkaline regions indicated red and blue, respectively. (C) The description of penetration and diffusion of an anticancer drug in the artificial tumor tissue. (D) A layout of the device, inlet ports for cell seeding, and Matrigel loading ports (panel 1). Scanning Electron Micrograph (SEM) of the cell transmigration region (panel 2). Cell migration, transmigration, and cell invasion area. Image of MDA-MB-435S cancer cells migrating through the region (redrectangle) shown on panel 2. (E) A micro cell culture analog with 3-D hydrogel culture of multiple cell lines that mimic metabolic functions of different organs and the tumor mass(Donald Wlodkowic et al, 2010).

E.1. Migration and metastasis:-

The Major cause of mortality in cancer patients is the nature of cancer cells to invade the neighboring tissue and consequently generate micro and macro metastases to secondary tumor sites (Joyce JA, Pollard JW,2009). For the treatment of metastatic and advanced tumors the conventional chemotherapy methods is largely inefficient and due to this new molecular therapies that target tumor metastatic potential are badly required (Joyce JA, Pollard JW,2009). Recently, a huge variety of microfluidic devise have been developed for the real time studies of (1) the invasion and transmigration of tumor cells by the endothelial basement membrane barrier (Chaw KC et al, 2007); (2) the dependency of cancer cells for the invasion in the 3 dimensional scaffolds on the embedded microvascular structures (Fischbach-Teschl C. et al, 2010); (3) 3D cancer cell motility in conditions of mechanical confinement in matrix-free environment (Irimia D, Toner M., 2009).

Conclusion:-

In this paper, we have summarized a few examples emphasizing on the achievements that are using human organ-on-chips, and the advantages of this unique approach over the existing culture systems used to examine the intercellular communication in vitro. As the synthetic biological approach offered by these organ-on-a-chip devices gives the ability to vary potential control parameters and also facilitating a window on molecular scale activities of living cells in an organ, they enable a very innovative form of human experimentation in vitro.

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