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In-vitro Cell Culture: A Promising Technology in the Advancement of Science and Research as a Solution to Human and Animal Health Issues

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ABSTRACT

From ancient Romans till late nineties, conceptualized first by Aristotle, it was believed that life evolved from non-living matter, the spontaneous generation theory. With the invention of microscopy, invisible life was for the first time visible as “animalcules”, that were later described as cells and the cell theory and theory of tissue formation came into limelight. In late nineties cell culture methods were discussed and *in-vivo* as well as *in-vitro* cell culture was achieved. *In-vitro* cell culture techniques were defined, improved, augmented and refined with the advancement in biological sciences. The applications of cell culture technology spanned through various fields, in pathology, microbiology, reproduction, physiology and production of biologicals, monoclonal antibodies, vaccines. *In-vitro* fertilization, embryo splitting and transgene delivery for augmenting fertility in human and animals, production of animal products, key biomolecules, proteins are some of the endowed applications of *in-vitro* cell culture technique. Cell culture technology has taken leaps and has become a key modelling system for large class of biotechnological interventions, clinical and industrial applications. Production of important hormones, growth factors, biomolecules and therapeutic agents are all blessings of *in-vitro* cell culture technology. *In-vitro* cell culture technology has entered into new era of development of organoids, organ chips, RNA/ DNA / recombinant vaccines, transgenic animals, tissue reconstructs *etc.* in pursuit of achieving better health care and food security. *In-vitro* cell culture technology has been playing a significant role in the global economy with huge market size in stem cell therapy, human infertility and production of therapeutic biomolecules. The *in-vitro* cell culture technology is promising, fast evolving, robust and matured technology with huge potential to revolutionize the fields of medicine, biological sciences and biomedical-engineering.

Key words: Biomolecules, Biotechnological tools, Cell culture, Organoids, Stem cell therapy, Tissue engineering.

In-vitro cell culture technology has evolved in late nineties and is fast growing technology, making its appearance in many fields of life sciences including biomedical engineering. Earlier the Aristotelian doctrine “spontaneous progression” was believed and unchallenged through centuries till late nineties. It was after the invention of microscopy that, enabled invisible life visible, changed the perception about the evolution of life and it was practically demonstrated that life originates from pre-existing life. In seventeenth century, it was Italy and Netherlands that played a vital role in the construction of microscopes and telescopes. With the advent of microscope, its applications extended in life sciences and Petrus Borellus described the use of microscope in medicine (Hajdu, 2002). It was Robert Hook (1665) and Leeuwenhoek (1677) who under microscope demonstrated microscopic structures of plants, insects, sponges, protists, bacteria *etc*. and made invisible microscopic world real and used the term “animalcules” for these microscopic structures that were later described “cells”. Lazzaro Spallanzani (1729-1799), Louis Pasteur (1822-1895) in their series of experiments demonstrated that life evolved from pre-existing life and maintained that microorganisms were in air and omnipresent.

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Cell theory

In the beginning of nineteenth century Matthias Schleiden (1804-1881) and Schwann (1839) put forth the cell theory and proposed that every structural unit of plant or animal tissues are made up of cells and the growth is actually the increase in the size and number of these cells (Hajdu, 2002). Shawan (1839) maintained that even highly differentiated

plants and animals are formed of cells and proposed one universal principle of development "the formation of cells". He also described three essential components of cells, the nucleus, fluid part and a wall/membrane. Virchow (1858) designated the cell as a fundamental unit of life as well as basal element of pathological processes and upheld that abnormal or pathological changes in the cells descent from a germ cell that leads to a disease (Deatherage, 1975).

In-vitro culture of bacterial cells

Thanks to the Louis Pasteur who optimized and developed first liquid culture media for propagation of microbes, thus microbiology got evolved in nineteenth century (Wainwright and Lederberg, 1992). Robert Koch developed solid culture media to have pure bacterial cultures which was not easy in liquid culture media (Sandle, 2011). In twentieth century, different specific/selective culture medias were produced to isolate different microbes, be aerobic or anaerobic or fungi or others (Arroyo and Arroyo, 1995). *In-vitro* culture technique of microbes was extensively used in medical pathology and microbiology for disease diagnosis and it still remains a fundamental reliable technique in diagnosis of infections. In 1960 antibiotic addition in the media to inhibit microbial growth opened a new window of antibiotic sensitivity test (AST) for different pathogens for effective control of diseases caused by microbes (Entis 2002). AST still stands a golden test for use of antibiotics against different pathogens. Preparation of curd, cheese and other fermented foods, an age old practice, principally involves cell culture technique (Katherine, 2022). *In-vitro* culture technique of microbes was utilized to produce vaccines in large scale. Mass production of bacteria through use of *in-vitro* culture technique is still a viable solution for production of number of bacterial vaccines in animal health care (HS, BQ, Anthrax vaccines). Bacterial cell cultures are also important source of production of primary metabolites like acids and alcohol and secondary metabolites like antibiotics. Bacterial cell cultures are also employed in biotransformation reactions (enzymatic, steroid), digestion of biodegradable wastes and microbial-mediated de-nitrification in waste water treatment (Daims *et al.,* 2001). Bacterial cell culture technique has been invariably useful in synthesis of recombinant proteins (therapeutic proteins) and production of biofuels (Mamma *et al.,* 1996; Abate *et al.,* 1996). *In-vitro* culture of certain microbes is being employed in agriculture for supporting plants in retrieving nitrogen (Franche *et al.,* 2009).

Interventions in naturally occurring microbial communities or production of synthetic microbial communities to exercise a specific function is a promising tool for innovating and enhancing their application in various clinical and industrial setups. Soil microbial communities can be propagated to degrade various pollutants like hydrocarbons (diesel) and polycyclic aromatic hydrocarbons, or prevent contamination of water from non-biodegradable pollutants, such as uranium, by catalysing their conversion to insoluble forms (Molina-Barahona *et al.,* 2004; Frutos *et al.,* 2010; Williams *et al.,* 2011). Microbial-mediated electricity generation through engineering, using a wide range of substrates including various industrial waste products (Kargi and Eker, 2007), Glucose or acetate (Zhang *et al.,* 2006) or cellulose (Ren *et al.,* 2007) or ammonium can be exploited to generate electricity (Lovley, 2006; Logan *et al.,* 2006).

In-vitro mammalian cell culture

In vitro cell culture technology has advanced significantly over last few decades and has attained robustness, reliability and maturity in its being used as a technology (Li *et al.,* 2010). After the discovery of cell theory, cell cultures have marked a major change in research. Wilhelm Roux was first to maintain living cells of the neural plate of chick embryos in saline buffer for few days in late nineteenth century (Rodriguez-Hernandez *et al.,* 2014). Loeb (1897) cultured the cells inside the body tissues by placing embryonic skin fragments of a guinea pig into the adult animal and observed growth of epithelial cells, however, it was not considered a strict cell culture. The American embryologist Ross (1907) was first to demonstrate *in-vitro* culture of frog nerve cells in a hanging drop method of *in-vitro* culture and the technique was reproducible. From here with this simple experiment, a new window of cell culture got opened to use *in-vitro* cell culture technique as a tool for evaluating cell physiology as well as production of monoclonal antibodies, vaccines and drugs.Carrel and Burrows (1911) successfully cultured tumours in plasmatic media by hanging drop method, of human, dog, chicken and rat. Carrel thereafter used serum instead of plasma along with varying concentrations of salt solution. Carrel (1912) added chicken embryo extract to culture media as supplement to culture animal cardiac cells. He also pioneered in sub-culturing and maintained growth for several months. Another great achievement in *in-vitro* culture of cells was design and development of synthetic medias for plant and animal cells (White, 1946; Fischer, 1947). Thereafter, the preparation of culture medias took the race and the "media - 199" (Morgan *et al.,* 1950), Earle's protein free media, Essential Medium (Earle *et al.,* 1943) and "Dulbecco modified Eagle's medium" were standardized and used in different cell cultures.

Gey *et al.* (1952) cultivated the cells, remarkably durable and fast dividing in every twenty hours, called HeLa (Henrietta Lacks) and propagated the poliovirus in them that lead to production of the Salk vaccine (Ambrose, 2017). HeLa cells are immortalised human cell line that is still one of the oldest human cell lines used in scientific research due to their prolificacy and durability. Owing to their unique characteristics, HeLa cells were quickly extended to various laboratories and pharmaceutical companies, making them one of the most prized resources for diverse studies.

The novel researches were made with the cells in *in-vitro* culture systems. It was in 1965, Harris and Watkins fused human and mouse cells and produced first hybrids mammalian cells (Harris *et al.,* 1965). Nobel Prize winners Kohler and Milstein (1975) accomplished the task of production of first monoclonal antibodies. The technique of introducing genes/ DNA in the cells cultured *in-vitro* was demonstrated by Graham and Van (1973). *In-vitro* cell culture provided researchers a novel opportunity to play with the mammalian cells. It was Wilmut and his colleagues (1997), who made a biological breakthrough in late twentieth century while producing world's first cloned sheep "Dolly".

In-vitro fertilization - conception in watch glass

The globally accepted technology (ART) "assisted reproductive technology" has been in use in humans and animals in a big way and its market value was USD 26.34 billion in 2022. This huge investment in the ART principally is blessing of *in-vitro* culture technology. As early as 1935 Pincus and Enzmann, at Harvard University, raised the possibility of development of mammalian eggs *in-vitro*. Roughly fourteen years later, oocytes were retrieved from women and exposed to spermatozoa *in-vitro* by Menkin and Rock (1948). However, Chang (1959) achieved births in a rabbit by *in-vitro* fertilization actually in a flask. At Johns Hopkins Hospital in USA, Robert *et al.* (1965), demonstrated fertilization of human oocytes *in-vitro*. The first IVF pregnancy in humans was reported by team of Carl Wood and John Leeton (De Kretzer *et al.,* 1973) that did not reach to term. However on July 25, 1978 the first ever IVF baby (test tube baby) "Louise Brown" was born in Oldham, England (Steptoe and Edwards, 1978). This assisted procreation was supported, reinforced by the experts in the field of microscopy, anatomy, embryology and physiology.

The oocyte *in-vitro* maturation and fertilization technique to produce embryos has been standardized with very good results in human and animals. Oocytes are retrieved from separated ovaries or intact ovaries and are matured in the laboratory to get ready for fertilization and then charged with sperms in the dish for fertilization and embryo development in the laboratory, which is followed by transfer into a surrogate mother. The IVF technique has been successfully employed in farm animals to tap high genetic merit of both males (*in vitro* fertilization with high merit semen or intracytoplasmic sperm injection) and females (oocyte retrieval, maturation and fertilization) (Fig 1). Ovum pick-up (OPU) technique has permitted retrieval of oocytes from prepubertal heifers and pregnant cows, thus increasing the lifetime productivity of elite females substantially and reducing the generation interval.

The *in-vitro* fertilization and birth of IVF babies have been achieved in almost all farm animals; the first successful birth of a bovine calf through IVF was achieved by Willet *et al.* (1951) at the University of Wisconsin.

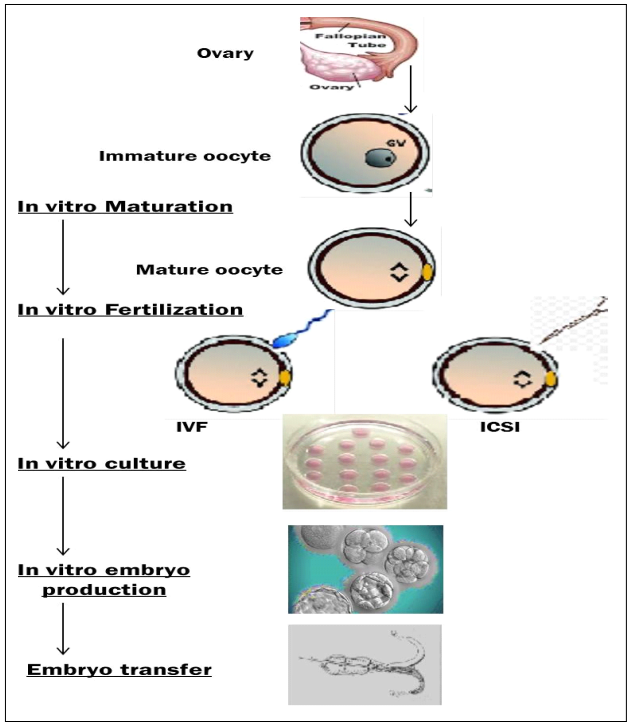

Fig 1: *In-vitro* production of embryos through IVF.

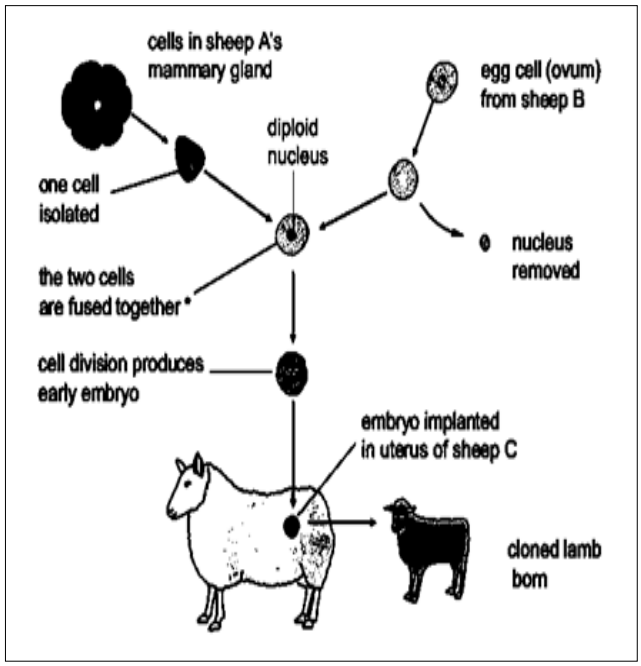

Fig 2: Creation of dolly sheep from somatic cell.

Brackett *et al.* (1982) produced first IVF calf in cattle; Cheng *et al.* (1986) in pigs and sheep, Goodrowe *et al.* (1988) in cats and Nagashima *et al.* (2015) in domestic dog. The technology of *in-vitro* production of embryos opened new windows for studying and exploring the process of fertilization, embryogenesis, karyotyping and human infertility. The IVF technology has potential to enhance the

productivity of food animals and conservation of endangered mammals (Bavister, 2002). With the advancement in allied techniques and cultural conditions, *in-vitro* fertilization and embryo production made it possible to have specific sex embryos, designed babies with specific phenotypic characters, cloned and transgenic animals. The *in-vitro* culture of human induced pluripotent cells with inherited disorders, have provided suitable in-dish platform for understanding the molecular mechanisms of a disease (Charis *et al.,* 2017).

Cloning

A copy or copies of an individual are produced in animals either naturally or artificially, when an embryo gets splitted to produce identical twins. The word "clone" has also been used to describe genetically identical offspring produced by nuclear transfer. The biggest breakthrough in cloning came in 1996 (Shampo *et al.,* 1996) when Wilmut and colleagues produced "Dolly sheep" by fusing a cultured adult somatic cell with an enucleated oocyte (Fig 2). Although still quite inefficient, cloning from adult cells offers the advantage of cloning genetically proven animals. Somatic cell cloning technique has now been successfully employed in almost all livestock species, including many laboratory animals and pets to produce animals of high merit of either sex, with desirable traits like growth, feed conversion efficiency, milk production or disease resistance. Somatic cell cloning technique has enabled species security as it requires any somatic cell DNA that is fused with enucleated oocyte and activated for reprogramming; the reconstructed cloned embryos are then cultured *in vitro* for a particular stage and transferred to synchronized recipients (Fig 3).

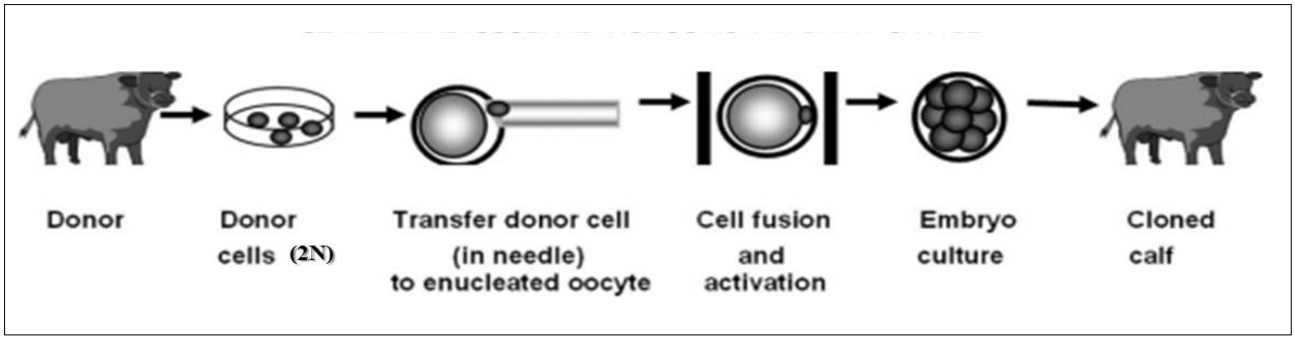

Fig 3: Cloning of animals from somatic cells.

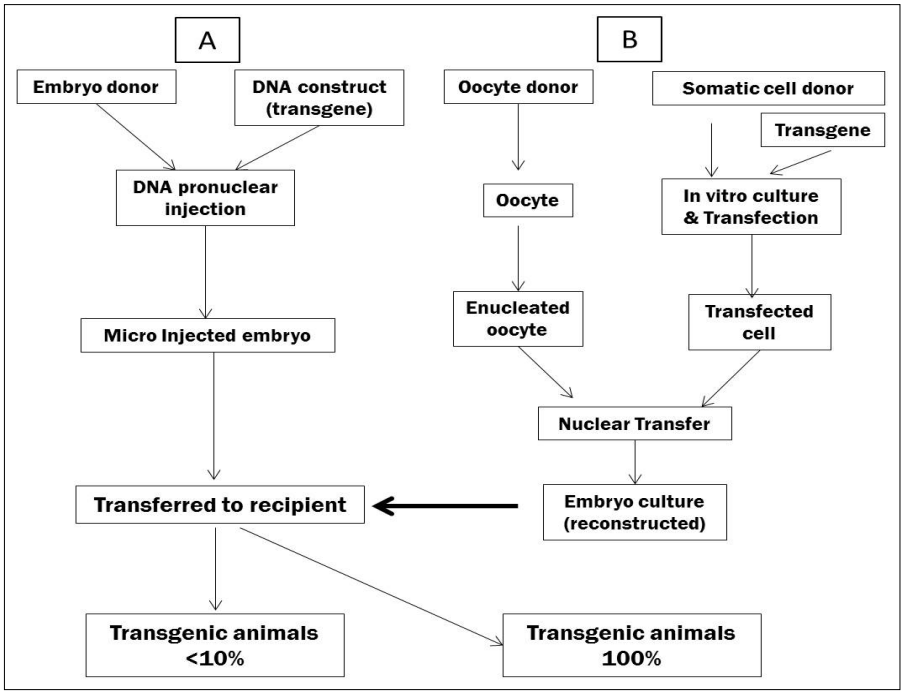

Fig 4: Production of transgenic animals.

Transgenic technologies

Allowed improvements that are currently not possible through traditional breeding schemes. An animal in which a foreign gene has been deliberately inserted into its genome is referred as transgenic animal. Transgenic sheep and goats have been produced that express foreign proteins in their milk. Recently a bacterial gene was expressed in the mammary gland to provide resistance against mastitis. The production of transgenic animals was first introduced by Gordon and colleagues (1980). Single gene insertions, deletions, or even modifications in gene sequence, using gene targeting has been possible with advances in cloning technology. Polly and Molly, two ewes born in 1997 (Schnieke *et al.,* 1997), were the first mammals to have been successfully cloned from an adult somatic cell and to be transgenic at the same time with a transgene, a therapeutic protein, human blood clotting factor IX (Fig 4). Pharmaceutical companies are looking dairy cows as bioreactors for production of various pharmaceutical products through a transgenic cow containing a gene for a particular drug that is expressed only in the milk.

In-vitro cell culture technology in understanding cell complexities

The cell culture technology has widened the approaches in the basic studies of cells. *In-vitro* cell culture has opened new areas of research for understanding cell metabolism, protein synthesis, gene transcription, translation, intracellular organelle activities, cell cycle, senescence, cell proliferation, differentiation and apoptosis (Andreeff *et al.,* 2000; Kourtis and Tavernarakis., 2009). *In-vitro* cell culture has given birth to "omics technologies" proteomics, genomics, transcriptomics. metabolomics that have unfolded the complexities of cell (Debmalya and Vasco, 2018). It was only possible through *in-vitro* culture technology to reach to the trails that work in microscopic entity like cell to perform variety of functions with temporal and spatial disposition. *In-vitro* culture of mammalian cells has also provided understanding of host cell culture state and through manipulation of media, feeding strategies using 'Omics technologies and mammalian bioprocess sciences, it is possible to design processes capable of regulating growth, death and other cellular pathways (Lewis *et al.,* 2016). *In-vitro* cell culture technology provides suitable and reliable platform to investigate the biology, physiology, biochemistry and metabolism of normal and diseased cells. It helps in understanding the interaction between wild-type cells and pathogenic agents like bacteria and viruses. *In-vitro* cell culture has provided a smart space for study of biomolecules, protein transport, assembly and disassembly of microtubules, cellular transformation, cellular interactions, adhesion and extracellular matrix interaction, gene expression, genetic analysis assays, infection, immortalization and modelling of cell lines for research (Andreeff *et al.,* 2000).

In-vitro cell culture technology in commercial production of therapeutic agents

In-vitro cell culture has provided a commercial setup for production of therapeutic proteins or biomolecules. In 1986, plasminogen activator (tPA), the first "recombinant tissue type" therapeutic protein was obtained in the culture of immortalized Chinese Hamster Ovary (CHO) cell line (Wrum, 2004; Lai *et al.,* 2013). Many other recombinant protein pharmaceuticals are expressed in CHO or other cell lines and it is estimated that about 60-70% of all recombinant proteins are produced in mammalian cells (Jayapal *et al.,* 2007; Lai *et al.,* 2013). A large list of products produced through recombinant technology include hormones like erythropoietin, FSH, LH, many blood clotting factors (F-VIII, F-IX), a good class of anti-inflammatory proteins or anti-immunoglobins. The production of recombinant proteins in mammalian cells has been taken to the next level of up-scaling in bioreactors.

Tissue culture vaccine production

In-vitro cell culture technology was employed in production of vaccines for use in human and animals alike. Vaccine against deadly disease Rabies was produced from human diploid cell cultures (HDCV) and from purified chick embryo cell cultures (PCECV). Researchers have demonstrated that growth of human pathogens like viruses in *in-vitro* cell cultures get attenuated and altered so that they can't replicate enough to cause illness in the human body, but capable enough to provoke an immune response. Taking leads from the study, a road map was chalked out for production of vaccines, using *in-vitro* cell culture technology, against many diseases like canine distemper, bluetongue, and rabies, Adenovirus vaccine, Rubella vaccine, Hepatitis A vaccines, Varicella (chickenpox) vaccine, Zoster (shingles) vaccine (Plotkin, 2006).

Cell culture setups were used to produce cloned vaccines using viral antigen genes and success has been achieved for transmissible gastroenteritis virus and Newcastle disease virus. Similarly, DNA plasmid vaccines were developed using *in-vitro* cell culture technology. DNA encoding viral antigens are first inserted into a bacterial plasmid, that is subsequently injected and taken up by the host cells within the cell nucleus, to transcribe the vaccine protein. Many vaccines were produced using DNA plasmid technique that include vaccine against West Nile virus infection in horses, avian influenza virus, Newcastle disease virus, canine parvovirus, bovine viral diarrhoea virus, foot-and-mouth disease virus, bovine herpesvirus-1, lymphocytic choriomeningitis virus, feline immunodeficiency virus, feline leukemia virus, porcine herpesvirus. Similarly instead of using DNA with vector to get expressed in cells as antigen protein, RNA has now been used to get translated in the cytoplasm in to an antigen protein and vaccines thus produced are referred RNA vaccines like Coronavirus vaccine. The flu viruses used in the cell-based

vaccines are grown in cultured cells of mammalian origin and Flucelvax Quadri valent is the only influenza vaccine, grown in a mammalian cell culture that is in use in the United States (CDC, NCIRD).

In-vitro cell culture has become indispensible for virus isolation and vaccine production because of cells being available in large numbers with choice of controlling cultural conditions and avoidance of experimental animals (Leland and Ginocchio, 2007). In pharmacology and toxicology cell culture applications include evaluating the effect of different drugs, drug-receptor interactions, phenomena of resistance, carcinogenesis, cytotoxicity, mutagenesis *etc*. (Naderi *et al.,* 2011). Cell cultures are now being used in drug discovery through adoption of new techniques like three-dimensional cell culture models (Smalley *et al.,* 2008) that vary from tissue engineering for clinical delivery through development of models for drug screening (Haycock, 2011).

Stem cell culture technology

In the early 1900's researchers recognized and acknowledged that the various type of blood cells like leukocytes, erythrocytes and thrombocytes do evolve from a particular mother cell 'Haemopoietic stem cell'. However, it was not until 1963 that research into stem cells grew out and for the first time self-renewing activities of transplanted mouse bone marrow stem cells were revealed (Becker *et al.,* 1963). Evans and Kaufman (1981) establishing a platform for pluripotent stem cells while harvesting mouse embryonic stem cells (mESC) from mouse embryos. Thomson *et al*. (1998), successfully established the world's first human embryonic stem cell line from the cells of spare embryos, which still exists today. The breakthrough discovery came when Takahashi *et al.* (2007) produced artificial counterparts from natural pluripotent stem cells in a dish. They demonstrated that introducing four pluripotency genes, Oct4, Sox2, Klf4 and c-Myc, in somatic cells can transform them in pluripotent stem cells and termed them as Induced Pluripotent stem cells (iPSC).

Stem cell therapy, commonly known as "bone marrow transplantation", has been in practice since past six decades now, pioneered by Thomas *et al. (*1957) who received a Nobel Prize in 1990. Stem cell culture has revolutionized the therapeutics and provided remedies to the diseases that were known to be untreated. Stem cell culture has also provided potential solution to regenerate the damaged tissue through paracrine activities and/ or through the trans-differentiation (Xu *et al.,* 2010) or through integration and fusion with the native host cells (Mansilla *et al.,* 2005). Stem cell therapy is gaining momentum in the contemporary world to address various ailments that were earlier considered irreparable and its applicability has also spread tremendously through creation of stem cell banks, to safeguard new-borns against the impact of more than 90 medical conditions. The global stem cell therapy market size valued at USD 13,266.8 million in 2022 that reflects the economic and therapeutic impact of stem cell technology. There are number of sources of stem cells either in adults or in foetuses or foetal appendages that are cultured *in-vitro* and directed to differentiate into particular type of cells for a particular treatment. Embryonic or foetal sources are preferred over adult sources and among them also mesenchymal stem cells are more acceptable because of their potential to differentiate into varied cell types (Amir *et al.,* 2023; Trounson and McDonald, 2015) and are immunogenically inert, thus more preferred for application in regenerative medicine (Fig 5).

Organoid culture

Stem cell culture technique has leaped into organoid culture and again offering a new platform to understand the cell to cell integration and functional regulation. Organoid culture is extremely fascinating culture system wherein 3D structured organs are developed that are self-organized tissue, simulating the key structural, functional and biological complexity of an organ, corresponding *in vivo* tissue (Zhao, 2022), hence providing a best research model for understanding its applications in variety of fields including drug discovery (Kim *et al.,* 2019), personalized companion diagnostics and cell therapy (Clevers, 2016). Organoids are typically derived from stem cells (pluripotent, tissue-resident, progenitor or differentiated) from healthy or diseased tissues as they critically maintain organ size, structure and function. However, they often lack stromal, vascular and immunological apparatuses (Yi *et al.,* 2021; Hofer and Lutolf, 2021) thus need to improve upon the understanding of organogenesis, angiogenesis. Since the organoids are tissue-engineered cell-based *in-vitro* models that copycat many aspects of the complex structure and functions of the corresponding *in-vivo* tissue, hence are more accessible and comparable for manipulation and in-depth biological studies than animal models (Clevers, 2016). Organoid models are now widely used in research on host-pathogen interactions. Tissue-derived organoids have been successfully used as transplantable material for tissue regeneration. Organoids of murine intestines, livers and pancreas have been successfully transplanted with restoration of organ function into mice (Peng *et al.,* 2018; Georgakopoulos *et al.,* 2020).

***In-vitro* cell culture empowers tissue engineering**

The term "tissue engineering" was earlier used in cases of surgical manipulation of tissues and organs or use of prosthetic devices or biomaterials up to the mid-1980s (Skalak and Fox, 1988). Tissue engineering is a multidisciplinary, integrated approach of scientists from biology, medicine and engineering field, to construct or reconstruct tissues, to repair, replace, maintain, or enhance the abilities of a specific tissue or organ by means of living cells. *In-vitro* cell culture technique has promisingly been applied in tissue engineering, to develop and establish cell growth so that in the future it is used as replacement tissue for damaged or malfunctioning tissue in the patients.

The technique uses living cells, biocompatible materials, growth factors, physical and mechanical loading factors and or combinations thereof to generate tissue-like structures with an ultimate goal to implant these tissue constructs in the body to repair or replace the injured and non-functional tissue. Tissue engineering has already shown success in clinical trials of skin, cartilage, bladder and trachea and this technology can fill a gap in the biomedical field. Tissue engineering provides suitable simulating platform for diagnostic screening, drug discovery, drug testing for efficacy and toxicity, bio-robotics as well as basic studies on tissue development and morphogenesis (Zorlutuna *et al.,* 2013). Tissue engineering technique involves the development of new concepts in cell culture technology using three-dimensional assortment of cells and the formation of an appropriate extracellular matrix, widely used as scaffold materials in tissue engineering (Sittinger *et al.,* 1996).

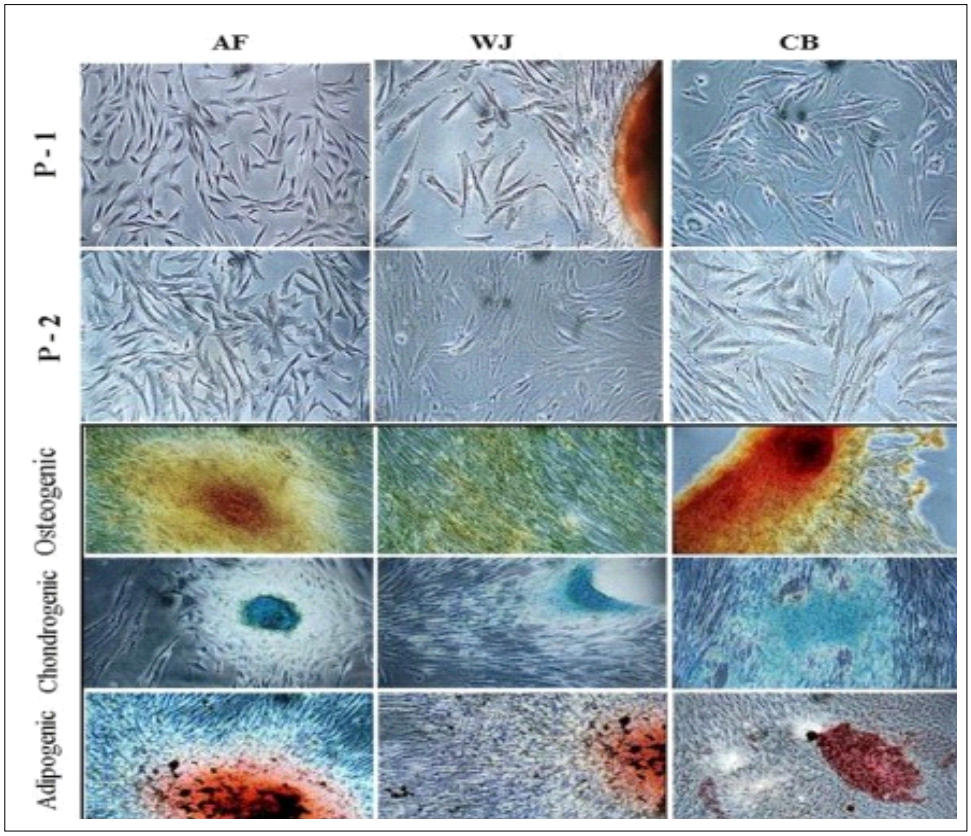

Fig 5: Stem cell culture and differentiation for use in regenerative medicine.

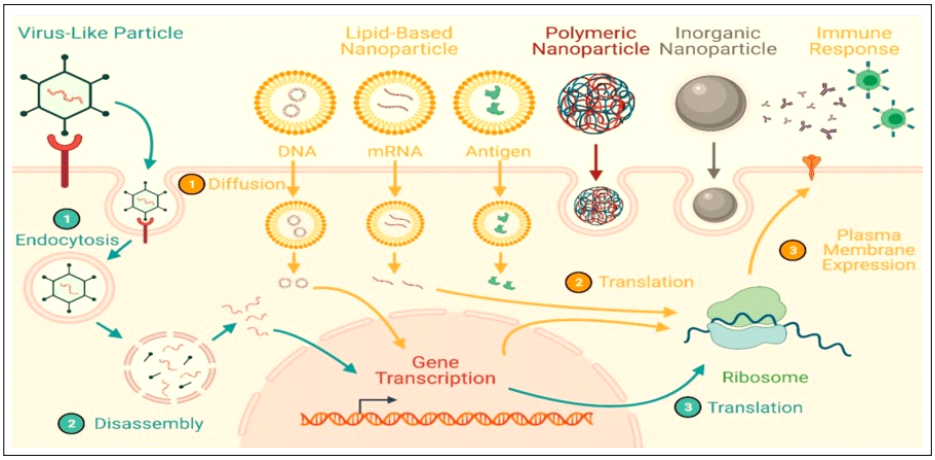

Fig 6: Application of nanoparticles in transgene delivery and vaccines production. (Taken from Megan *et al.*, 2021).

In-vitro cell culture in Organ-on-chip

Organ-on-chips (OoCs) again an advanced *in-vitro* cell culture technology that comprises engineered or natural miniature tissues grown inside microfluidic chips, to simulate human physiology in *in-vivo* environment. The chips are designed to have strong grip over cell micro-environment and maintain tissue-specific functions. Organ-on-chip provides a micro-scale platform to attain a higher level of control over the microenvironment, tissue-life support system and a means to directly observe behaviour of cells and tissues. Advances in tissue engineering along with microfabrication, OoCs have gained great interest as a future experimental platform to explore human pathophysiology and the effect of therapeutics in the body (Whitesides, 2006; Bhatia and Ingber, 2014).

In-vitro cell culture integrates with nanotechnology

Nanotechnology offers a world of possibilities in diverse industries and scientific investigations. Nanotechnology has the potential to collaborate with stem-cell therapy due to unique characteristics of nano-materials to improve the efficiency of cell-based therapy (Corradetti and Ferrari, 2016). Integration of two disciplines, nanotechnology and stem cell sciences, opens a new window to identify the role of molecular apparatus in stem cell differentiation mechanics. Nanoparticle have been developed for use in stem cell isolation, lineage differentiation, stem cell imaging and tracking, regenerative medicine and tissue engineering (Abdal *et al.,* 2018; Zhao *et al.,* 2011). Very recently, the role of nanoparticles in gene transfer, drug delivery, therapeutics, diagnostics and imaging has revolutionized the biomedical field. Induced pluripotent cells were often used in transgene delivery, but it was not easy to transfect iPSCs, however, nanoparticles made it easy to deliver transgene into the cells and offeres a simple, highly efficient and robust approach with low cytotoxicity, low cost, biodegradability and non- immunogenic in transfection. BioNTech/Pfizer and Moderna both developed mRNA COVID-19 vaccines using lipid-based nanoparticles that ensured effective delivery of mRNA for spike protein translation into the cells that subsequently would trigger an immune reaction (Diaz and Zing, 2020; Shin *et al.,* 2020). Many other types of nanoparticles may be engineered and exploited for delivery (Fig 6).

CONCLUSION

Cell culture technology has emerged as an essential tool in the progress of science as a solution to many human and animal health issues. Cell culture technique is a reproducible technology that can be one of the most extensively used potential tool in research and development of pharmaceuticals, vaccines, hormones, biomolecules and tissue constructs besides augmenting animal production for food and nutritional security.

Conflict of interest

No potential conflict of interest is reported on behalf of all authors of this manuscript.

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