# TISSUE ENGINEERING
# -An Era of Regenerative Medicine

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**ABSTRACT**

Annually, 1.2 million people on an average need transplantation for end stage organ failure. The, then successful transplants are contingent upon immunosuppressants. This is probably due to allogeneic source of transplants. What if the autologous source gets appreciated? The answer to this query is the field of Tissue Engineering, which is an approach to repair or replace the damaged tissues and organs with their mimics. This multidisciplinary topic of Regenerative Medicine raises certain questions on the *in vivo* functioning of the native and the transplanted one. To conciliate these, the autologous cells are made to grow in the native extracellular matrix (or so), thereby making them vie for signaling like the natives do. Since autologous cells are preferred, the chance of immune rejection and search for donor is not applicable. Moreover, this approach provides a way to defeat the uncurable disorders like Muscular Dystrophy, by production of healthy skeletal muscles *in vitro*. Albeit theoretically feasible, the set of methods bound to regenerative medicine lack mechanical integrity and efficacy desired. Despite this, thousands of surgical procedures are performed everyday to replace or repair tissue that has been damaged through disease or trauma.

**KEYWORDS**

ESCs, MSCs, IPSCs, CRISPR/Cas systems, Bone Tissue engineering, Collagen, NF scaffold, BDNF, EGF, ADSCs, Artificial Trachea, *In-vitro* meat, Artificial Skin.

## INTRODUCTION

Tissue Engineering is a field of Regenerative Medicine, collaborating the minds of scientists, physicians, and engineers, to construct or reconstruct the human tissues or organs. The term was first used by a bioengineer, Yuan-Cheng Fung in 1985, in a proposal for funding the Center for Engineering of Living Tissue at the University of California, San Diego **(1)**. The purpose of tissue engineering owes to assemble the functional constructs that restores, maintains, or improves the damaged tissues or organs. This approach ascribes to Cells, Scaffold and Growth Factors (Regulatory Signals) **(2).**

The history dates back to 15th century, when Fra Angelico gave a pictorial presentation of Tissue Engineering, entitled, 'The Healing of Justinian'. In her painting, Justinian appears to be slept when Saints Cosmas and Damian enter his chamber. They replaced his corrupted leg with a healthy one **(3)**. In 1597, Gasparo Tagliacozzi, professor of Surgery and Anatomy at the University of Bologna, described a nose replacement in his work 'De Custorum Chirurigia per Insitionem' [The Surgery of defects by Implantation] **(4)**. W.T.Green in 1970s, a pediatric orthopedic surgeon at Children's Hospital, undertook many experiments to generate a cartilage using chondrocytes. Though unsuccessful, he gave the idea of use of biocompatible materials (now called, Scaffolds) **(5)**. Dr. Burke and Dr. Yannas generated the first artificial skin in 1981, by growing the dermal fibroblasts on collagen matrix **(6)**.

## CELLS

Tissue Engineering emphasizes more on the use of autologous stem cells, rather than allogenic ones **(7)**. Embryonic Stem Cells (ESCs) have largest differentiation and proliferation capacity, but its use is banned in many countries due to legal and ethical issues **(8)**. However, Adult stem cells provide an alternative, with major challenges being safe and effective approach. Mesenchymal stromal/stem cells (MSCs) are non-hematopoietic, multipotent, immune privileged cells with potential proliferation and differentiation capacity; and without risk of teratoma formation **(8) (9) (10)**. Commonly exploited MSCs are Umbilical Cord derived MSCs, Bone marrow derived MSCs and Adipose derived MSCs **(11)**. The major drawback of MSCs is that these cells cannot differentiate to form any type of cells. To overcome this, iPSCs (induced/-ible Pluripotent Stem Cell) approach is favored, which can induce the formation of cells of any lineage **(12)**. But if even one cell remains undifferentiated, it can give rise to teratomas. CRISPR/Cas systems (Clustered Regularly Interspaced Short Palindromic Repeats/ CRISPR associated protein) provide a simpler, reliable and economical alternative to the use of exogenous growth factors, for inducing differentiation of stem cells to the desired lineage. These prokaryotic, nucleic acids targeting machineries are in use nowadays **(13)**. Bone tissue engineering involves the use of ESC, embryonic SC derived MSC or multipotent human amniotic fluid derived MSC. ESC derived MSC are more homogeneous than ESC, and have higher

proliferative capacity than MSCs, and are commonly used. Human MSCs have the potential to differentiate into bone and fat tissues, and are well exploited in cartilage tissue engineering. Human Dental Pulp stem cells (hDPSCs) differentiate into odontoblasts and form dentin, and this forms the basis of dental tissue engineering. Bone-marrow stem cells (BMSCs) differentiate into SMC (smooth muscle cells) to form Bladder and Vascular Tissues **(14)**.

SCAFFOLD

Once the autologous stem cells are selected and cultured, the next step involves the fabrication of scaffolds. Scaffolds are the substances provide a structural support for the cells to proliferate. Biocompatibility, bioresorbability, biodegradation, non-toxicity and high mechanical integrity are the attributes of the scaffold used in tissue engineering **(15)**. Nevertheless, scaffold architecture with requisite porosity and pore geometry should be conducive to cell size and cell morphology respectively **(16)**. Natural scaffolds may have protein origin like collagen or polysaccharide origin like alginate. Synthetic scaffolds can be hydrogels like Polyethylene glycol (PEG) or ceramics like Tetracalcium phosphate (TCP) **(17)**.

Collagen is the major component of natural ECM, and is an excellent scaffold to support cell growth and cell communication, as it has natural binding sites for cells. Though it has low Young's modulus, but can be increased by cross-linking or by conjunction with other synthetic polymers **(18) (19)**. Hydroxyapatite/TCP represents the example of collagen-synthetic polymers used in bone tissue engineering **(20)**. Polymers used as resorbable surgical sutures are the best examples of synthetic polymers (scaffolds). They are coated with bioactive materials, and are easily bioresorbed and biodegraded **(21)**. Polyethylene glycol (PEG), Poly vinyl alcohol (PVA) and Polyacrylic acid (PAA) are the hydrogels found useful typically for the soft tissues. These water soluble polymers are crosslinked to form an insoluble network. Since they lack any natural binding site, it is desired to coat these gels with some adhesive proteins, so as to mimic the natural ECM **(22)**.

Since the natural polymers can act as a good ECM, thus the native ECM can too be utilized as a scaffold for regenerative medicine. This approach is referred as Decellularization and Recellularization approach. Decellularization is associated with the lysis of cells, followed by their removal, thereby leaving an acellular scaffold. The lysis is done by either mechanical, physical or chemical methods **(23)**. After decellularization, recellularization is done by seeding the cells, either by injecting them or by perfusion. The appropriate mixture and number of cells are to be taken into considerance during cell seeding. For example, an adult liver (70 kg) homes about $2.8*10^{11}$ hepatocytes **(23) (24)**. The major limitation of this approach is immunogenicity and pathogen transmission **(25)**. Another promising technique for Scaffold formation by referencing a model or prototype is Rapid Prototyping **(26)**. This includes the group of 3D printing techniques that involves the quick fabrication of a scale model of a physical part using CAD/CAM technologies.

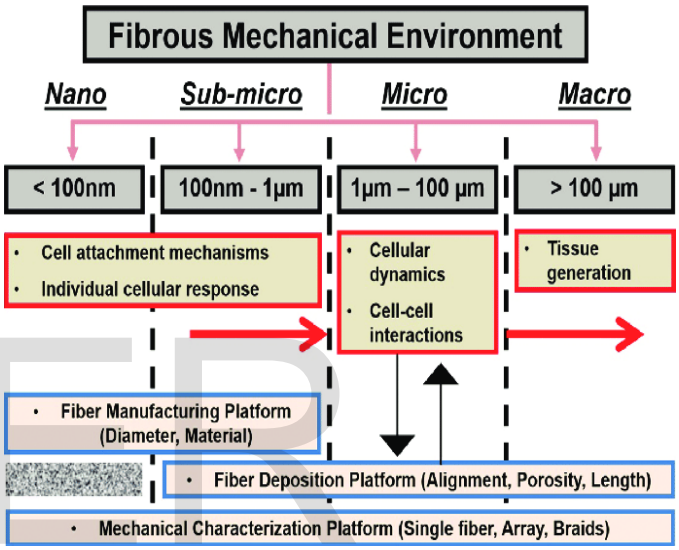


**Fig 1**: Hierarchial order of scaffolds according to their porosity and mechanical environment **(27)**.

Moreover, there is a hierarchial order of scaffold as shown in figure1 **(27)**. The high surface to volume ratio of Nanofibrous (NF) scaffolds increases cell adhesion, cell proliferation, and differentiation more proficiently. Due to additional evaluated properties like porosity, pore size, elastic modulus and permeability, NF scaffolds have been found to have a wide range of applications in tissue engineering, from drug delivery to wound healing (skin grafts) **(28)**. Polyurethane NF, collagen NF, chitin NF scaffolds in skin grafting; Poly tetrafluoroethane (PTFE), Poly(L-lactide-co-ε-caprolactone) scaffolds in blood vessel grafting are immensely used **(29)**. The major limitation of using NF scaffold is their low mechanical integrity to mimic the native ECM. Various fabrication methods for NF scaffolds have been developed so far. Commonly used ones are:

1. Phase-Separation
   Polymer is dissolved in a proper solvent at low temperature, followed by solvent exchange. Its

successive freeze-drying gives NF architecture. Phase separation depends on the solvent for dissolution, solvent for exchange and the temperature **(25) (29)**.

2. Electrospinning
   Polymer is given high charge by applying high voltage to it. The collector plate is linked to the negative potential,that allows the formation of NF scaffold. Voltage, collector and distance between two, are the factors that regulate electrospinning **(29) (30)**. It typically forms 2D nanofibrous sheets **(25)**.
3. Self-Assembly
   Silk protein is found to undergo self-assembly and form micelles. In order to stabilize those micelles, NF structures are produced. Molecular mobility, charge, hydrophilic interactions and concentrations are the parameters to control this method **(29) (31)**.
4. Melt-Blowing
   Polymers and granules of a particular size are allowed to spin through hot air. This hot polymer is then allowed to pass through the cooling air, and the melt blown fleece is collected. Melt blowing technique is regulated by the size of granules and the extent of hot and cool air **(29) (32)**.

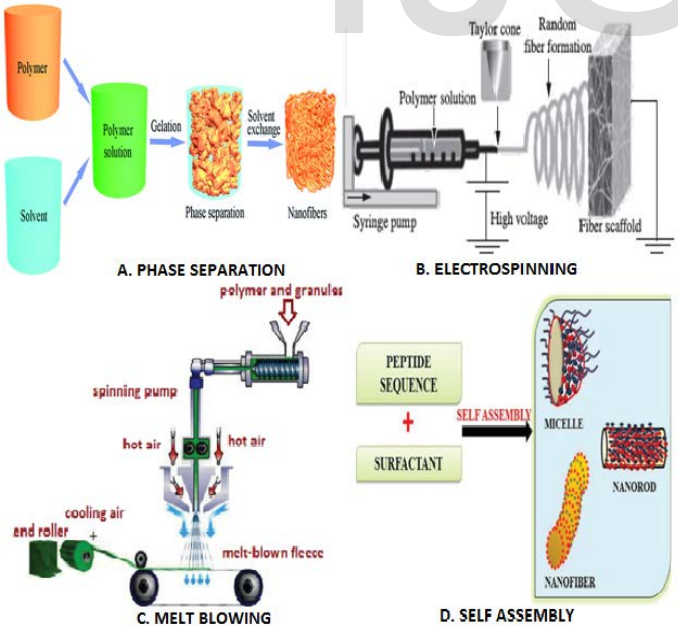


**Fig 2**: Fabrication methods of NF scaffolds; Phase separation (A), Electrospinning (B), Melt Blowing (C) and Self- Assembly (D) **(29) (30) (31) (32)**.

GROWTH FACTORS

Inorder to mimic a native tissue, the cells must proliferate and differentiate in a biochemical manner. To carry out such metabolism, some bioactive signals are required to be delivered, while some are required to be withdrawn (probably, by inhibition) from the system as shown in figure3 **(33)**. Neurotrophins like NGF (Nerve Growth Factor), BDNF (Brain-derived Neurotrophic Factor), NT-3 (Neurotrophin-3), etc. regulate the neuronal functions. TGF (Transforming Growth Factor), FGF (Fibroblast Growth Factor), IGF (Insulin-like Growth Factor) are the factors required for chondrogenesis. To maintain liver functions, factors like EGF (Epidermal Growth Factor), aFGF (acidic Fibroblast Growth Factor), HGF/SF (Hepatocyte Growth Factor/ Scatter Factor) are required **(34)**. Albeit high levels of BMP-2 increases the risk of cancer formation,VEGF (Vascular endothelial Growth Factor) and BMPs (Bone Morphogenetic Proteins) hold the basis of bone tissue engineering **(35)**.

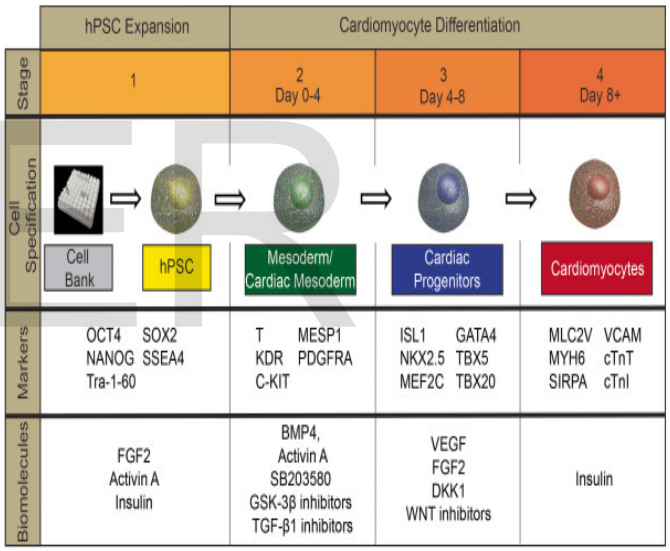


**Fig 3**: This depicts the role of bioactive molecules in differentiation of human PSCs to Cardiomyocytes **(33)**.

Growth factors constitute the pivot class among the bioactive signals, and are known to have chemotactic, mitogenic, morphogenic, apoptotic, metabolic effects and so on **(36)**. These effects depend upon the concentration of factors, their exposure time and phenotype of the target cells. *In vivo* growth factors are released by healthy cell lines to direct the maturation (of other cells) and repair (of injured tissues) **(37)**. Instructions for cell infiltration, cell proliferation and matrix synthesis (deposition and organization) are provided by growth factors. This subsequently leads to angiogenesis formation and finally, the tissue formation **(37) (38)**. Secreted growth factors can act via autocrine, paracrine or telecrine pathways.

Delivery methods of growth factors include: Burst release (for porous scaffolds, hydrogels, etc.); Sustained release (for microspheres, nanoparticles, etc.); Delayed release (for well-designed microspheres, core-shell vehicles, etc.); Pulse-like release (for well-designed composites, multifunctional vehicles, etc.); Continuous release (for fibrillar collagen, alginate gel, etc.); Pulsatile release (for hydrogels, on-off delivery systems, etc.) **(39)**. Loading efficiency, preservation of GF bioactivity, methods of immobilization, simplicity and safety are the considerations to be made during delivery **(40)**.

## APPLICATIONS

Though unsuccessful on many trials, this field of regenerative medicine has provided solutions to many problems.

### Artificial Trachea

On 9th April 2013, Dr. Paolo Macchiarini and his team performed an operation on a toddler of two and a half year, Hannah Warren. Hannah was born with a rare, congenital abnormality due in which her trachea failed to develop and since birth, she was on ventilator. The team used her bone marrow mono nuclear cells (BMMNCs) and mixed with a synthetic scaffold of PLGA [poly (lactic-co-glycolic acid)] in a bioreactor, so that the cells could attach to the polymer. The scaffold was designed using Hannah's chest as a template **(41)**. This created an artificial trachea that Hannah was transplanted with, but unfortunately she died after 3 months. The doctors maintained that the operation involved the surgery of Toddler's oesophagus that never healed properly **(42)**. However, it has been reported that seven out of nine patients, who received artificial trachea transplants by Dr. Paolo, had lost their life **(43)**.

### Artificial Skin

Dr. Burke (from Massachusettes General Hospital) and Dr. Yannas (from MIT Harvard) team announced the formation of artificial skin in 1981. They seeded the dermal fibroblasts on the collagen matrix **(6)**. Later, Dr. Howard Green used this technique and transferred the sheets of keratinocytes onto the patients with burns **(6)**.

### *In-vitro* Meat

Adipose tissue derived SCs (ADSCs) are the multipotent stem cells that are found in adipose tissue of animals. They can be further trans-differentiated into myogeneic, ostogeneic, chondrogeneic, adipogeneic cell lineages, using appropriate growth factors. Using collagen, cellulose, alginate or chitosan as a scaffold, *in vitro* meat is produced. However, its production is prohibited in many countries, due to ethical issues **(44)**.

### Artificial Skeletal Muscles

Human iPSC derived 3D artificial muscle serves the features of normal skeletal muscles, and are believed to provide a solution to the incurable disorder of muscles, Muscular Dystrophy. Isogeneic vascular-like networks and motor neurons can be developed within artificial muscles, thereby mimicking the native muscles **(45)**.

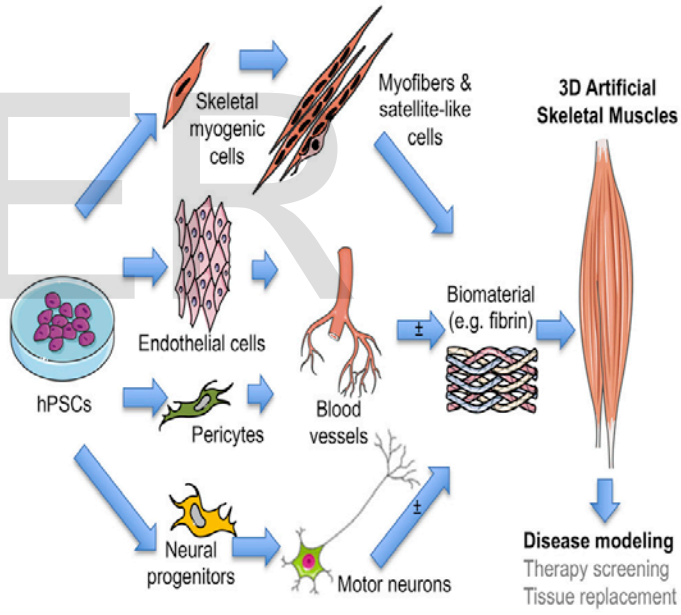


**Fig 4**: Formation of artificial skeletal muscles from hPSCs, as a disease model, therapy screener and tissue replacer **(45)**.

## CONCLUSION

Tissue Engineering is a developing field of Regenerative medicine. Generating healthy tissue (or organ) requires an interplay between cells, scaffold and biomolecules. However, certain ethical issues, low mechanical integrity, less efficacy and cost are the limiting factors for this technology. Moreover, the tissue engineering products are yet to be proven successful on humans. Fortunately, with

the advents in this technology, it is possible to envisage the formation of intricate organs, and even the whole human body.

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