Stem Cells and Extra Cellular Matrices: Applications in Tissue Engineering

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The ability to artificially simulate the 'mechanical' niche, broadly termed as Extracellular Matrix (ECM), of the bone marrow determines success for stem cell growth, architectural organization and differentiation viz. tissue engineering. The advent of various natural and synthetic polymers has greatly influenced tissue engineering. The focus of the review is on various artificial niche simulations, ECM, scaffolds such as hydrogels, electrospun nano and micro fibers, bone-strengthening scaffolds and tissue infills. The utility of the ECMs in the treatment of various medical conditions including bone and cartilage tissues, nervous tissues, spinal cord and tendon tissues as well as wound healing, along with the ability of some ECMs in entrapment of elusive cell secretomes will be discussed. The future of tissue engineering has indeed got a new lease of life with polymer scaffolds and it is feasible that certain goals, thought of impossible so far, may become possible.

INTRODUCTION

Stem cells, or for that matter all cells, for formation of viable and functional tissues, require interaction with their specific niche. The niches comprise the biochemical niche, including, soluble factors, cytokines, chemokines, growth factors and several other factors. Further, the mechanical niche, the acellular compartment, provide scaffold for the biochemical niche. In a natural environment, both these niches together play a crucial role in cell growth, differentiation and fate determination, besides a very critical role in functional organ/organelle formation. A major hurdle in the area of tissue engineering is to understand and simulate the complex niches. The primary hurdle is creating a three dimensional (3D) atmosphere for cell growth, which will allow not only mimicking tissue architecture, but also creating a gradient of biochemical components in cell–cell interactions.

Our ability to artificially simulate this complex and the co-ordinated/regulated environment will be a major leap in ability to understand and thereby direct stem cell fate, propelling the cells into targeted functional tissue formation, the basic goal of tissue engineering.

The current mini-review will focus on the

mechanical niche component, broadly termed Extracellular Matrix (ECM) component to simulate the natural tissue composition. The understanding of the biochemical fraction of the niche and modus of choosing a material close to the natural niche are vast topics, and thus not dealt with here.

The advent of biocompatible polymers has enhanced the ability to perform grafting, implanting, delivery and substitution of non-functional biological tissue with function reinstating artificial options. These are fast emerging potential alternatives to autografts and allografts, in short supply and carry risks of disease transmission. The scaffolds are used to engineer various soft connective tissues such as skin, ligament, muscle and tendon, as well as vascular and neural tissues. And for advanced cell therapies, the ECMs aid in long-term cell culture in a 3D system, enhance cellular propagation and act as an efficient system for targeted cellular delivery.

Scaffolds

A large part of what can be achieved in tissue engineering is dependent on the types and functional abilities of the various extracellular matrices/scaffolds available. A multitude of scaffolds are currently available for cellular growth, cellular/non-cellular delivery, regeneration of damaged tissue and replacement of degenerated tissue. Many more are being added to the list every day.

The currently available scaffolds fall largely into two broad categories, natural and synthetic; subcategorized into degradable and non-degradable (Dandayuthapani *et al*., 2011). These properties largely depend on the composition, structure and arrangement of the constituent macromolecules, broadly characterized into ceramics, glasses, polymers and several others. Of these, natural and some biodegradable or non-biodegradable polymers are most commonly preferred for tissue engineering purposes, referred to as 'biomaterials'. Some of the naturally occurring polymers are silk, collagen, gelatin, fibrinogen, elastin, keratin, actin and myosin. Naturally occurring polysaccharides such as cellulose, amylose, dextran, chitin, and glycosamino glycans are most favoured for preparation of scaffolds/matrices due to the high levels of biocompatibility (Ratner *et al*., 2004).

Synthetic materials often mimic the physicochemical and mechanical properties of biological tissues, thus enhancing the ability to stand-in for and repair damage to functional tissue. Besides, synthetic polymers are highly valued for the ability to manipulate porosity, tensile strength, degradation time and mechanical characteristics. Additionally, reproducibility, mass production, structural uniformity and long shelf life render them cost effective (Gunatillake *et al*., 2006). Some of the commonly used polymers such as polylactic acid (PLA), polyglycolic acid (PGA), polylactide-*co*-glycolide (PLGA) and

polyhydroxyalkanoate (PHA) copolymers are most widely used polymers for tissue engineering (Chen *et al*., 2002; Ma, 2004). Hydrogel scaffolds are important as also array of polymeric scaffolds/matrices available to tissue engineers. Some of the natural hydrogels are collagen, fibrin, alginate, chitosan; while the synthetic counterparts include PLA and perfluoroalkoxy (PFA) derived polymers, poly(ethylene glycol) (PEG) derivatives and poly(vinyl alcohol) (PVA) (Behravesh *et al*., 2003; Bryant *et al*., 2004; Eyrich *et al*.,2007; Kim *et al*., 2004; Kong *et al*., 2003; Schmedlen *et al*., 2002; Solchaga *et al*., 2002; Suh *et al*., 2000; Wallace *et al*., 2003). Recently, our group has successfully demonstrated the use of Puramatrix hydrogel (Becton Dickinson, New Jersey, USA) for creation of a 3D equivalent of bone marrow (BM) niche *in vitro* (Sharma *et al*., 2012).

Of the many classes of synthetic materials used, polymeric composites are fast evolving as in demand scaffold materials, to mimic ECM-like environment. Consequently, these serve as cell propagation sites as well as cellular delivery modules. These also act as the mechanical component of the stem cell niche, thereby contributing actively to tissue formation.

The fabrication of successful 3D scaffolds is a complex phenomenon and involves special attention to factors such as macro/microstructure, interconnectivity, surface charge and area, porosity and pore size, biocompatibility and mechanical strength. The ECMs most amenable to these functions are the electrospun matrices. These electrospun matrices/scaffolds allow flexibility of scaffold formation in the micro and nanometer range. The advent of 3D scaffolds that mimic the nano-architecture of biological tissues has opened up a host of avenues and possibilities in tissue engineering (Vasita *et al*., 2006). The mechanical properties and wide range of degradation patterns available for polymeric scaffolds are of great importance in the quest for nano-tissue engineering scaffolds/devices (Sokolsky-Papkov *et al*., 2007). One of these nanodevices is the electrospun nanofibre matrix, which shows great morphological similarities to various biological extracellular matrices. These are characterized by continuous fibres, high surface to volume ratio, high porosity and manually variable poresize. Electrospun nanofibres may be tagged with various biocompatible/bioactive molecules, thereby increasing the possibilities of cellular adherence and growth. This enables supply of necessary chemical cues for growth of specific cell types. The tensile strength of the scaffolds allows use in cell delivery in *in vivo* experiments (Kumbhar *et al*., 2008). Most interestingly, the tensile strength of the scaffolds are remarkably similar to skin and marginally lower than human cartilage, demonstrating that nanofibre scaffolds are
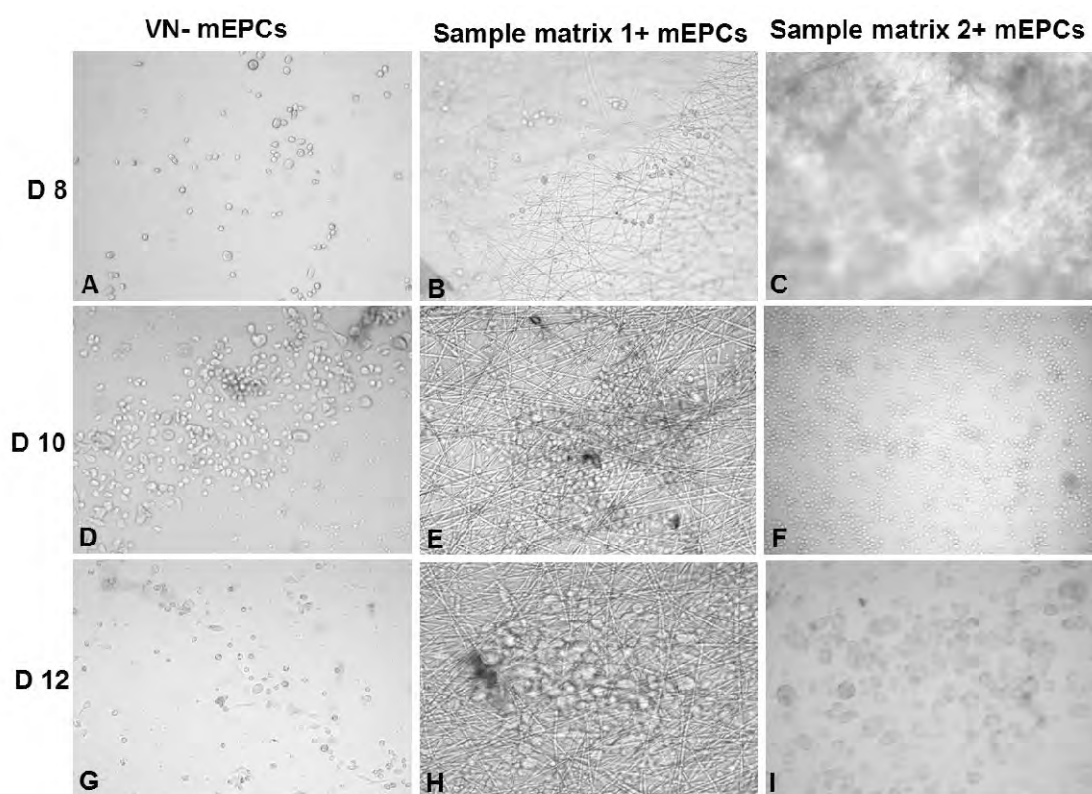
Figure 1: Light microscope image depicts two preparations of electrospun nanofiber matrices (3D systems) supporting varying degrees of endothelial progenitor cell (EPC) growth from day 8 to 10. Vitronectin is the standard 2D control, which also supports EPC growth, albeit to a markedly lesser extent.

candidates for implantation or for regeneration of cartilages (Fischer *et al*., 2012; Shin *et al*., 2006).

The use of these biofriendly polymeric materials has added to the vistas for the types and extent of tissues regenerated, particularly for stem cells, given their higher requirement for niche regulated support. The 3D architecture of ECMs/scaffolds allows enhanced cell growth as well as tissue like intercellular interactions. The thickness of the matrix component influences cell–cell dynamics and eventual tissue application. As represented in the microphotograph, sample matrix 1 is thinner than sample matrix 2 (details withheld so as to not compromise patent filing) and consequently shows lower cellular growth from d8 to d10 (Fig. 1). A benefit to a thinner matrix enhances the visualization potential.

In context, it is evident that certain biological symptoms and disorders have benefited more than others due to the usage of nanofibrous and other ECMs/scaffold induced tissue applications. Several of the disorders are related to skin, bone, cartilage, liver, heart valves, arteries, bladders, pancreas, nerves, tendons, spinal cord, corneas and other soft

tissues (Boyan *et al*., 1999; Diedwardo *et al*., 1999; Eaglstein *et al*., 1998; Germain *et al*., 1999; Mayer *et al*., 1997; 2000; Mohammad *et al*., 2000; Oberpenning *et al*., 1999; Tziampazis *et al*., 1995).

Bone

Osteoporosis is induced by impaired balance between the activities of cellular constituents of the bone, osteoblasts and osteoclasts. ECMs facilitate formation of osteoblasts from non-osteo lineage stem cells, such as mesenchymal stem cells (MSCs). Yoshimoto *et al*. (2003) successfully cultured and expanded MSCs on polycaprolactone (PCL) scaffolds and propelled them into osteogenic lineage under dynamic culture conditions for four weeks. Interestingly, cell-embedded matrices maintained the size and shape of the original scaffold (Yoshiomoto *et al*., 2003). Since osteoporosis make bones fragile, bone grafts are important. Mineralized polymeric nanofibrous composites have been successfully employed as materials for bone grafts (Ngiam *et al*., 2009*)*. Although bone formation is a crucial step in regeneration, it alone does not suffice for larger bones, such as femur performing crucial weight bearing functions. Complete regeneration of these bones has been a hurdle. However, applications of ECMS/scaffold techniques have made this feasible. For the purpose of load-bearing tissue engineering, a novel biodegradable nanocomposite porous scaffold comprising a b-tricalcium phosphate (b-TCP) matrix and hydroxyl apatite nanofibers has been developed by a method combining gel casting and polymer, resulting in bone formation with enhanced capacity for load bearing (Ramay *et al*., 2004). Recently, a new composite material consisting of mesoporous bioactive glass (MBG) and concentrated alginate pastes were used for fabrication of hierarchical scaffolds by 3D plotting. This scaffold structure contains well ordered nano channels, micropores and controllable macropores beneficial for bone tissue engineering applications and drug delivery (Luo *et al*., 2013).

Sponge techniques

Apart from the usual type of scaffolds, natural polymers such as silk have been tested for their bone-building ability. Studies on the effect of primary or multiple silk coating revealed efficacy of these natural polymers in improving mechanical and biological properties of biphasic calcium phosphate (BCP) scaffolds, including *in vitro* evaluation of the osteogenic response of human MSCs (hMSCs) on the coated scaffolds. The multiple silk coating proved to be a simple, yet an effective technique for reinforcement. This could also be applied to other types of ceramic scaffolds with similar microstructure to improve osteogenic outcomes (Bogush *et al*., 2009; Li *et al*., 2013). With current developments in the ECM technology, it has

become possible to integrate ECM components with non-degradable synthetic components, including beads. This technological advance is useful in bone morphogenesis. hMSCs entrapped in alginate hydrogel loaded with ECM coated beads, contributed to enhanced bone formation *in vitro*, indicating that engineered ECM may be employed in a minimally invasive manner to direct formation of bony tissue (Bhat *et al*., 2013). Current techniques have also facilitated slow release of bone formation related proteins, such as bone morphology protein-2 (BMP-2), by complexing them with various ECM components such as dermatan sulphate (DS), hyaluronic acid (HA) hydrogels. *In vivo* studies on rats demonstrated that HA-hydrogel delivered BMP-2 precomplexed with glycosamine glycans (GAGs) induced twice the amount of bone formation compared to controls (Kisiel *et al*., 2013).

Vascular engineering

The idea that ECM may be able to influence microvasculature of endothelial cells and promote angiogenesis is not a new one. Feng *et al*. (1999) demonstrated that ECM environment could regulate human dermal microvasculature and promote endothelial cells into higher microvessel formation (Feng *et al*., 1999). The advent of nano-fiber technology has amply benefited the field of blood vessel formation, vascular grafts etc.

Currently, different types of stem cells are used for formation of blood vessels including MSCs and endothelial progenitor stem cells (EPCs). Hashi *et al*. (2007) used nanofibrous grafts for regeneration of vascular grafts and successfully employed the antithrombogenic properties of BM-MSCs for tissue vascularization. Coronary artery smooth muscle cells, also capable of forming blood vessels, have been successfully employed for long term vascularization using poly-L-lactic-co-ε-caprolactone nanofibrous scaffolds (Dong *et al*., 2008). Cell numbers often demarcate the efficacy of an available graft; thus increasing the need for 3D scaffolds to enhance cellularization (Williamson *et al*., 2006). Mun *et al*. (2012) have used 3D electrospun nanofiber poly-L-lactic acid (PLLA) matrices for small diameter vascular grafts, thereby enhancing functionality of the graft (Mun *et al*., 2012). The poly-caprolactone-polyurethane (PCL-PU) composite scaffold was developed by wet spinning PCL fibres which form the luminal surface, then electro-spinning porous PU onto the back of the PCL fibres to form the vessel wall substitute. This was successfully used as a device for small diameter vascular grafts and showed high capability for endothelial cell attachment and proliferation to form a monolayer with strong platelet/endothelial cell adhesion molecule-1 (PECAM-1) expression and cobblestone morphology (Hau-Min *et al*., 2013).
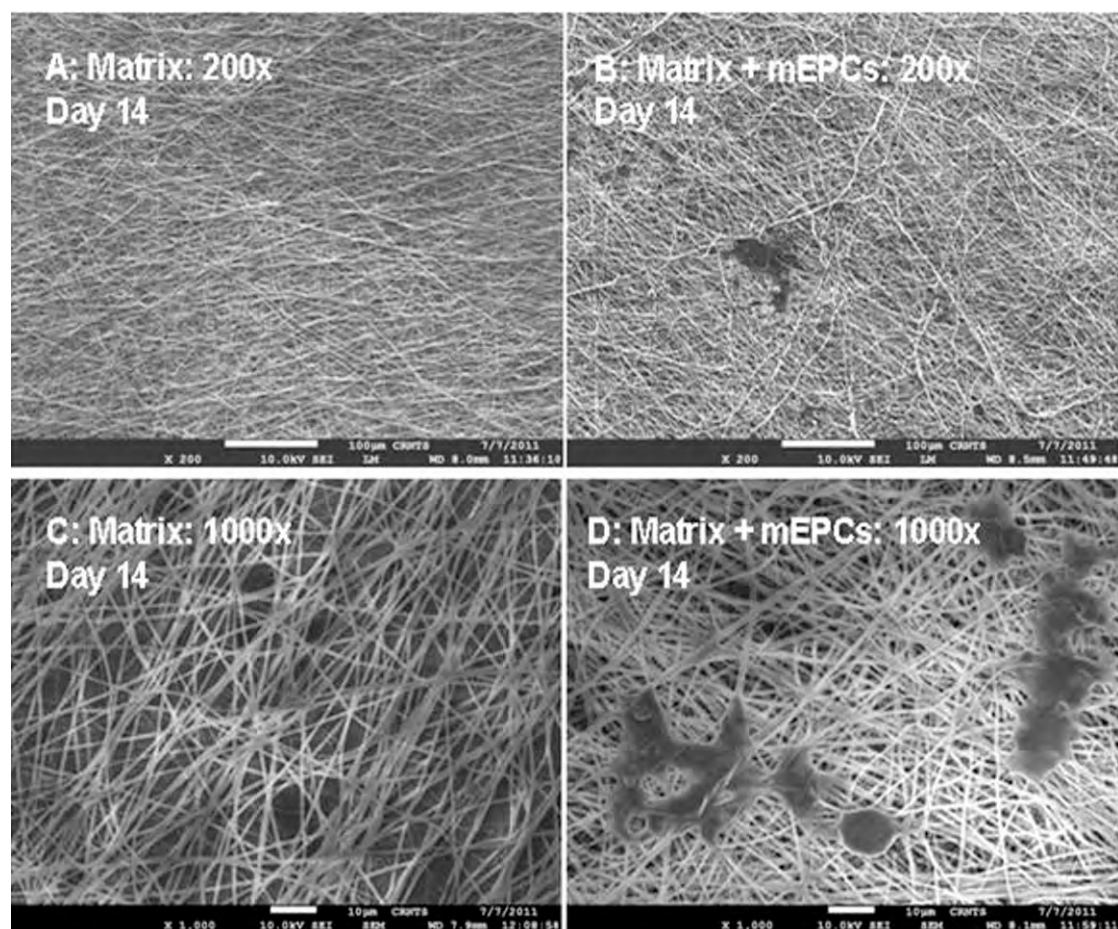
Figure 2: Scanning electron microscopy image depicts PCG matrix supporting murine EPCs for a long term culture, while maintaining cellular morphology. Images of PCG matrix without EPCs (A) and with EPCs (B) are illustrated (Magnification 200x). Images of PCG matrix without (C) and with (D) m-BM-EPCs at day 14 in culture (magnification 1000x).

Nerve, tendon and spinal cord tissue engineering

ECMs/scaffolds have benefitted the field of nerve tissue engineering. Several different types of polymers have made their mark for development of nervous tissues including hyaluronan-gelatin, etc. Yang *et al*. (2004) developed a porous polymeric nanofibrous scaffold using a biodegradable polymer, PLLA, for *in vitro* culture of nerve cells. Since then PLLA has been widely used in tissue engineering for a variety of purposes besides nerve tissue engineering. Similar polymers and derivatives, such as microspheres, have also been deployed with advantage. Polyphosphoester miscrospheres or polymer bound natural biomaterials, have been used with success for sustained release of biologically active nerve growth factors leading to enhanced growth of nerve cells (Sun *et al*., 2009; Xu *et al*., 2002). Tendon neogenesis has also benefited from

development of these scaffolds (Xu *et al*., 2013). Spinal cord engineering has benefited greatly by hydrogel type of tissue infills, which cover the sheath and eventually contribute to spinal cord regeneration (Macaya *et al*., 2012).

Wound healing

The basic problem in using stem cells for wound healing applications, bandage style, is the cell loss due to flow away mechanisms, reducing efficacy of the transplanted cells. For this purpose, a matrix that can function both as a cell growth substrate and cell delivery scaffold will be most efficacious. The technique of electrospinning various polymers into nano/microfibrous scaffolds has revolutionized the field of wound repair using stem cells. In an interesting study, human adipose tissue derived stem cells were seeded onto a silk–fibrin–chitosan scaffold. The cells not only enhanced wound healing in a soft tissue injury mouse model, but also demonstrated differentiation into various lineages linked to wound healing, such as fibrovascular endothelial and epithelial cells in the restored tissue (Altman *et al*., 2009). Studies have also revealed that self assembling peptide nanofiber scaffolds accelerate wound healing in a bioengineered Human Skin Equivalent (HSE) tissue model that enabled wound re-epithelialization to be monitored in a tissue that recapitulates molecular and cellular mechanisms of repair in human skin (Lahiji *et*

al., 2000). Similar studies showed successful results in burn wounds (Meteroja *et al*., 2013). In our laboratory polycaprolactone-gelatin (PCG) electrospun nanofibrous matrix is in use for long term and enhanced EPC culture, as a 'ready-to-use' EPC delivery scaffold for treatment of diabetes induced impaired wound healing (Fig. 2). The application of the matrix embedded cells enhanced the rate of EPC growth about four times as the controls; while application of the PCG embedded EPC patch onto wound sites in diabetic mice, enhanced wound healing rate significantly, indicating the tremendous potential of such treatments for similar medical conditions (Fukuda *et al*., 2006).

ECM assisted co-culture systems

Cell co-culture systems are used in several fields of biomedical sciences. Consequently, advances in the techniques on the interface of tissue and biological engineering contributed to several types of tissue culture systems requiring co-culture, or multi-culture of various cell types. A simple interface system using chitosan was devised as early as 2000, for human osteoblasts and chrondrocytes (Nagata *et al*., 2002). Cartilage tissue engineering is a complex subject. A co-culture system comprising MSCs and chondrocytes has proved promising for development of other types of cells. Its benefits were recently harvested for creation of hypoxia, deemed to be beneficial for cartilage development

(Schneider *et al*., 2008). In an ingenious approach, Fukuda *et al*. (2006) created micro patterned cell co-cultures using two ECMs deposited one on top of the other. The system demonstrated the potential benefit of growing more than one type of cell(s) (Meng *et al*., 2009). Collagen matrices have been known to retard, and perhaps increase overall longevity of rat pancreatic islets of Langerhans (Bakota *et al*., 2011). Our recent data (unpublished data, personal communication) indicated successful culture of three cell types, in varying proportions using a simple, electrospun nanofibrous matrix. The results implied promise of harvesting and harnessing the properties of elusive secretomes (unpublished data, personal communication). This approach emphasizes importance of multiple cell culture engineering over simple ECM regulated cultures. The approach may reveal new routes of stem cell and primary cell co-cultures.

Cellular secretomes

Recently, it has been demonstrated that not only the cells, but the cellular secretomes can be harnessed for therapeutic purposes. Recently, several groups have harnessed the MSC secretome for treatment of cardio-vascular disease (Wang *et al*., 2011). Several other studies follow similar patterns. Taking a lead from this secretome dependent therapeutic approach, Bakota *et al*. (2011) devised an injectable multi domain peptide nanofiber hydrogel as a delivery agent for stem cell secretome. At a concentration of 1% by weight, this peptide forms extensive nanofibrous network, resulting in a physically crosslinked viscoelastic hydrogel. The hydrogel undergoes shear thinning and quickly recovers 100% of its elastic modulus when the shearing force is released, making it ideal for use as an injectable material (Kanitkar *et al*., 2013). The group also used secretome pre-conditioned peptide nanofibers for renal protection following acute kidney injury (Ranganath *et al*., 2012). Contextually, harvesting the cell secretome is a tedious task typically involving collection of conditioned media and enrichment of active components, which may result in loss of several labile molecules like proteins and peptides. The nanofibrous matrices with small pore sizes may be employed for entrapment and easy harvesting of these cell secretomes with hydrogel-like consistency (unpublished data, personal communication).

The cellular secretomes may possibly mimic the exact biochemical component of the stem cell niche and hence special efforts should be directed at understanding the composition and functionality of the 'secretome'. Indirectly, the artificial mechanical component may allow us to 'trap' and analyse the biochemical component.

The current overview highlights the applications of various ECM/scaffold induced regeneration by promoting cell growth and/or

permit cell delivery. The examples and citations give an idea of the extensive application in the field of disease biology and the benefits accrued. The resourcefulness and efforts of the scientific community in the field has created a range of scaffolds, with respect to materials, thickness, pore size, degradability, shapes such as sheets, cylinders, fibres, micro/mega spheres etc., to choose from depending on the specific application. The future of tissue engineering has indeed got a new impetus with polymer scaffolds and multiplied the implications in biomedical applications.

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CONFLICT OF INTEREST

The authors claim no conflict of interest.

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