

Status of tissue engineering and regenerative medicine in Iran and related advanced tools: Bioreactors and scaffolds

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ABSTRACT

Because of increased need to tissue and organ transplantation, tissue engineering (TE) researches have significantly increased in recent years in Iran. The present study explored briefly the advances in the TE approaches in Iran. Through comprehensive search, we explored main TE components researches include cell, scaffold, growth factor and bioreactor conducted in Iran. The field of TE and regenerative medicine in Iran dates back to the early part of the 1990 decade and the advent of stem cell researches. During past two decades, Iran was one of leader in stem cell research in Middle East. The next major step in TE was application and fabrication of scaffolds for TE in the early 2000s with focused on engineering bone and nerve tissue. Iranian researchers extensively used natural scaffolds in their studies and hybridized natural polymers and inorganic scaffolds. There are many universities and government research institutes are conducting active research on tissue-engineering technologies. Limitations to TE in Iran include property design and validation of bioreactors. In conclusion, in the last few years, fields of tissue engineering and regenerative medicine such as stem cell technology and scaffolds have progressed in Iran, but one of the biggest challenges for TE is bioreactors researches.

Keywords: Iran; Tissue Engineering; Cell; Scaffold; Signal; Bioreactor

1. INTRODUCTION

Because of increased mean life expectancy, Road traffic injuries (RTIs) and disabilities resulted from Iran-Iraq

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war the loss and damage of tissue is a great challenge for public health of Iran [1].

Road traffic injuries (RTIs) are a major global public health problem [2-4]. In 2005, 500,000 Iranians injured annual in road traffic crashes, and the death and injury rate is the highest of any country in the world for which reliable estimates are available [5,6]. In other study, Towhidpour and Amiruddin indicated that in 2009, road fatality rates were 30.96 per 100,000 population while middle-income countries had 19.5 per 100,000 population roads traffic fatality rates. This study demonstrated that the rate of road injuries between 2007 and 2009 increased by 10% when compared with 2005 [7].

8-year of the Iran-Iraq war victims are another great challenge for public health of Iran. During the war (1980 to 1988), physical and psychological disability of approximately 500,000 people rapidly increased need to tissue and organ transplantation [8]. However, low availability of compatible donor's severely limited transplantation of tissues or organs for these patients [9]. Thus, new strategy and therapy concepts for repair of diseased and damaged tissue are required. Therefore, biomedical research activities such as TE in Iran have significantly increased in recent years. Various sources of funding especially through the Iranian government in the recent years have been influential in increasing TE /biomedical researches.

2. TISSUE ENGINEERING

TE is an alternative to organ transplantation. A more commonly used definition of TE, as coined by Langer and Vacanti, is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve function of a tissue or a whole organ" [10,11]. Many researchers have defined six components

for TE, namely, cells, scaffolds, bioreactors, stimulation (mechanical etc.), cell-cell contact and chemical factors (growth factors, etc.)

3. PAST, PRESENT AND FUTURE OF TE IN IRAN

3.1. History of Ancient TE and Medicine in Iran

Iran has a long history of scientific achievements and prior to Islam, Iran was a leader in mathematics and astronomy. Furthermore, regenerative medicine has a long history in Iran. As reported in archaeological literatures, the earliest artificial eye (5000-year-old golden artificial eye) found by an Iranian archaeologist in Shahr-i-Sokhta. Shahr-i-Sokhta (Burnt City) is in the Sistan desert on the Iranian-Afghan border [12] (**Figure 1**).

The field of TE and regenerative medicine in Iran dates back to the early part of the 1990 decade and the advent of stem cell researches, *i.e.* the production of stem cell line. The Royan Institute is Iran's leading stem cell research center. The institute established in 1991 and approved by the Iranian Ministry of Health in 1998. The institute has been main forum presenting stem cell research activities for recent years [13].

3.2. 2000 to Present

The next major step in TE progress in Iran was application and fabrication of scaffolds for TE in the early 2000s. The earliest attempt carry out TE effort was reported in the Iranian Polymer Journal in the year 2000 [14]. It was not until 2000-2010, however, that one began to see more and more researches in this emerging field. This is when many of the pioneers in TE research began to make their mark [15-18]. It is important to consider that the focus of Iranian researchers was on engineering bone and



Figure 1. The earliest artificial eye was found by an Iranian archaeologist in Shahr-i-Sokhta, Iran.

nerve tissue, by an integrated use of stem cells and bio-material scaffolds. This is due, at least in part, to high rate of trauma and spinal injuries during Iran-Iraq war [2-8] and the advent of stem cell research.

In attempts to engineer other tissues, such as skin, cartilage, and hepatic and even in efforts to recreate neo tissues, several promising studies conducted. Recently Iranian researchers have extensively used scaffolds for TE [19-23]. Long-term goals of Iranian researchers are to engineer tissue grafts for application in regenerative medicine, develop enabling technologies for stem cell research, and design high-fidelity models for studies of tissue development, remodeling, and disease. Therefore, the number of Iranian academic and industrial groups studying TE is rapidly increasing, leading to more journal articles on TE (**Figure 2**).

There are many universities and government research institutes in Iran, such as Royan Institute [24-26], Amir-kabir University [19,20,27-30], Pasteur Institute of Iran [31-34], University of Tehran [20,35], Tarbiat Modares University [20,36-39], Shaheed Beheshti University of Medical Sciences [40,41,43,45], Tehran University of Medical Sciences [42,44], National Institute for Genetic Engineering [46], Islamic Azad University [47-49], Iran University of Science and Technology [50,51] and Iran Polymer and Petrochemical Institute [52,53] are conducting active research on the development of artificial organs based on tissue-engineering technologies.

3.3. Future

The global effort will write the future history of TE and regenerative medicine in Iran. As we look into this future, in some cases, the development of TE will made the advances in treatment or therapy will be made through the development of tissue engineering.

In following sections, we review researches based on TE component in detail.

4. CELLS

In this section, we will focus on a number of different sources and most cells used by Iranian researchers for tissue repair and regeneration each with its own advantages and disadvantages.

4.1. Mature Cells

Isolated cells from tissue biopsies can potentially use for TE. Mature cells are the most desirable with regard to immunological compatibility. However, they are probably not the best cell source for tissue repair. Because they are differentiated and post-mitotic cell and some cell types have the tendency to de-differentiate during *ex vivo* cultivation [54]. However, since a study suggested that intra-epidermal injection of dissociated epidermal

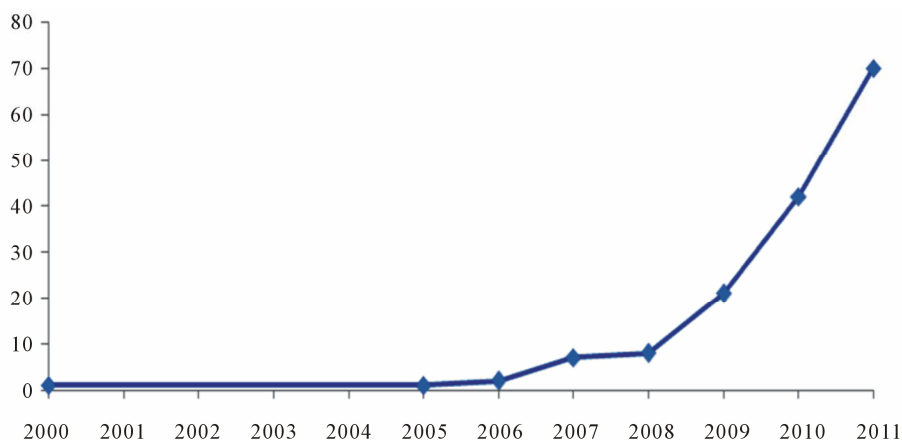


Figure 2. Trend and number of published articles related to TE researchers in Iran that indexed in Pub Med database from 2000 to June 2011.

cells into the lesions of stable vitiligo patients, is an effective, simple and safe therapeutic option for stable vitiligo lesions [55], several researchers in Iran investigated TE with mature cells [25,32].

4.2. Stem Cells

Stem cells are precursor cells that possess certain characteristics. These characteristics serve to distinguish them from other cell types. Definition of Stem cells done by two properties: self renewal and differentiation [56]. Stem cells can be classified according to developmental potential and origin. Depending to measures of stem cell potency, the cells may be classified into tot potential, pluripotential, multipotential, and unipotential cells. Depending on their origin, stem cells are classified into embryonic stem (ES) cells, embryonic germ (EG) cells, embryonal carcinoma (EC) cells, trophoblast stem (TS) cells, and foetal stem cells [57]. Since Iran is one of leader in stem cell research in Middle East here, we review most stem cells used by Iranian researchers.

4.2.1. Adult Stem Cells (ASCs)

Adult stem cells are undifferentiated cells, which reside in differentiated tissues. Many adult tissues contain the stem cells that can be isolated. They are responsible for growth, tissue maintenance, regeneration, and repair of diseased or damaged tissues. In general, there are two distinct types of ASC within the bone marrow the hematopoietic stem cell (HSC) and bone marrow stem cell (BMSC) [58].

4.2.1.1. Mesenchymal Stem Cells (MSCs)

There are some reports that when compared, the differentiating potential of these cells for osteogenic capacity is greater than Unrestricted Somatic Stem Cells (USSCs) and Adipose stem cells (ASCs) [42-68]. A clinical study

revealed that MSCs loaded to the biphasic scaffold (beta-tricalcium phosphate/hydroxyapatite), may enhance bone formation in the maxillary sinus area for sinus elevation [44]. In other clinical study patients with unilateral alveolar cleft, treated with the composite scaffold of demineralized bone mineral and calcium sulphate (Osteoset) loaded with mesenchymal stem cells (MSCs). Computed tomograms showed regenerated bone after 4 months [41].

Mohammadi and colleagues reported that culture of MSCs in Nanofibrous poly (epsilon-caprolactone)/poly (vinyl alcohol)/chitosan hybrid upregulated the expression of early and late markers of osteoblastic differentiation [67].

The other study investigated the effect of transplantation MSCs induced into endoderm and early hepatic cells in streptozotocin (STZ)-induced diabetic mice, and suggested that MSCs are suitable candidates for cell-based therapy of diabetes mellitus [63]. Further study revealed that the engineered nanofibrous scaffold Poly (epsilon-caprolactone), collagen and polyethersulfone) is a conductive matrix, which supports and enhances MSC development into functional hepatocyte-like cells [39]. Furthermore, in a clinical experiment, human mesenchymal stem cells (MSCs) from eye conjunctiva stromal cells were differentiated into dopaminergic neurons. The MSCs were treated with general neurogenic medium expressed of dopaminergic neuron genes such as TH, Ptx3, and Nurr1. Therefore, was concluded that CJMSCs might be a suitable and available source for cell transplantation therapy for the central system diseases such as Parkinson's disease (PD) [66]. In other in vitro neurogenic study of MSCs, the two-stage induction protocol resulted in transdifferentiation of BMSCs into GABAergic cells with synaptic release upon stimulation [68]. Also Soleimani and colleagues reported that poly L-lactic acid (PLLA) could be used to support neurogenic and

hinder dopaminergic differentiation of conjunctiva mesenchymal stem cells (CJMSCs) *in vitro* [37]. Meanwhile,

Ahmadbeigi and colleagues for first time reported spontaneous, non-tumorigenic immortalization of BM-MS-C in rabbits [62]. Therefore, Ghaedi and colleagues proposed that hMSCs may serve as an effective platform for the targeted delivery of therapeutic proteins to cancer sites [60]. The other study revealed that applying appropriate loading treatment on hMSCs could enhance proliferation capability, and produce functional SMCs for engineered tissues [29]. Mohajeri and colleagues improved mechanical properties of collagen to enable mesenchymal stem cells to positively improve their proliferation and differentiation [38].

4.2.1.2. Adipose Stem Cells (ASCs)

Adipose tissue is one of the richest sources of MSC's [64]. When compared to bone marrow, there are more than 500 times more stem cells in 1 gram of fat when compared to 1 gram of aspirated bone marrow. Studies showed that surfaces containing HGF co-printed with ECM proteins can be used to differentiate mesenchymal stem cells such as ASCs into hepatocyte-like cells [61].

4.2.2. Embryonic Stem (ES) Cells

Pluripotential embryonic stem cells derived from the inner cell mass of the blastocyst. Embryonic stem cells do not exist in the body. When cells are isolated, they can be massively expanded in the laboratory, while maintaining their pluripotency (self-renewal), then differentiated.

Researchers examined the differentiating potential of human embryonic stem cells (hESCs) into hepatocytes. Results revealed that the differentiated cells give rise to cells displaying morphological features, gene expression patterns, and metabolic activities characteristic of hepatocytes. Authors proposed that hESCs may provide a source of differentiated cells for treatment of liver diseases [15].

Several Iranian researchers differentiated ESCs into neural cells in defined adherent culture by retinoic acid and concluded that ESCs seeded in scaffold such as poly-L-lysine-coated PLGA and collagen can be as a model system for nerve tissue engineering, thus may provide a new approach for the repair of spinal cord injury [24, 26,69].

4.2.3. Placenta Derived Stem Cells (Amniotic)

Up to ten times as many, stem cells can be harvested from a placenta as from cord blood for differentiation. Niknejad and coworkers reviewed properties of the amniotic membrane for potential use in tissue engineering [43].

4.2.4. Unrestricted Somatic Stem Cells (USSCs)

These non-hematopoietic stem cells in cord blood (CB) appear not to be simply MSCs. The frequency of these stem cells is also higher in CB than in adult bone marrow, probably due to the very rapid growth. USSCs have the potential to differentiate into mesodermal, ectodermal as well as into endodermal cells. Thus, Iranian researchers investigated differentiating potential of the cells into mesodermal cells such as bone. Therefore, the capacity of fabricated scaffolds for bone formation was investigated *in vitro* using USSCs under osteogenic induction. The authors introduced a new combination of stem cell-scaffold constructs with desired characteristics for application in bone tissue engineering [19,20,34,35]. In other attempt for investigate differentiating potential of the cells into endodermal cells, Hashemi and colleagues showed that hepatocyte-like cells differentiated from USSCs on the PCL nanofiber scaffold can be candidate for tissue engineering and cell therapy of hepatic tissues [23].

5. SCAFFOLDS

5.1. Definition and Roles in TE

The officially accepted definition considers a scaffold as a "support, delivery vehicle or matrix for facilitating the migration, binding or transport of cells or bioactive molecules used to replace, repair or regenerate tissues" [70].

The functions of the scaffold are to provide a three-dimensional (3D) cell culture template that can induce seeded cells to regenerate full tissues and to degrade at a known, controlled rate. Thus, the important roles played by scaffolds TE are to mimic the functions of native ECM, at least partially [71].

5.2. Property and Types

The scaffold for TE should facilitate cell adhesion and promote cell growth. Furthermore, the scaffold should be biocompatible, biodegradable, highly porous with, mechanically strong, and malleable [72,73].

Many types of biomaterials and scaffolding can be used to make scaffolds for TE. Here we review most biomaterial and scaffolding used by Iranian researchers.

5.2.1. Pre-Made Porous Scaffolds for Cell Seeding

They can be classified into two categories according to their sources, namely natural (e.g. collagen) and synthetic biomaterials (e.g. polyglycolide).

Due to their biocompatibility, relative abundance and commercial availability, and ease of processing, Iranian researchers extensively used natural scaffold in their studies [21,22,46,69]. Hatami and colleagues investi-

gated generation of hESC-derived neural precursor as neural-like tubes in adherent and feeder-free hESC. They reported that hESC-NPC transplants in collagen scaffolds, promote recovery in injured rat spinal cord [22]. Similar results reported by Baharvand and colleagues [69]. In other attempt, Pajoum Shariati and colleagues developed a culture method for propagation of keratinocytes and fibroblasts isolated from human neonatal foreskin. They used modified method of freeze gelation for fabrication of relevant biodegradable and biocompatible collagen-chitosan porous scaffold with improved biostability. Findings these procedures introduced a potential source of living skin for grafting *in vivo* [21]. Also, it was reported that collagen gel can be used as a bio-scaffold for the production of engineered sperm analogues and proposed that [46].

Synthetic scaffold are largely divided into two categories biodegradable and nonbiodegradable. Some non-biodegradable include polyvinylalcohol (PVA), etc. Some synthetic biodegradable polymers are the family of poly (α -hydroxy esters) such as polyglycolide (PGA), polylactide (PLA), etc. In spite, synthetic biomaterials have better controlled physical and mechanical but because of difficulties in attachment and growth on these materials, biocompatibility becomes the major issue. Several studies investigated TE approaches with the polymers [18,23,31,33,47-49,53,65,74,77]. Shariatpanahi and coworkers investigated the effect of grafting N-vinyl-2-pyrrolidone (NVP) onto polyvinyl alcohol (PVA). PVA scaffolds were prepared by freeze-thaw and progen (glycerol) methods. Cell attachment was optimal for the introduced scaffolds [18].

In other study biocompatibility of β -TCP/HDPE-UHMWPE nanocomposite as a new bone substitute material was attempted by using highly purified human osteoblast cells. Results indicated that the nanosized β -TCP/HDPE-UHMWPE blend composites could be potential scaffold, which is used in bone tissue engineering [31]. Heidarkhan Tehrani and colleagues hypothesized to be able to quantify the efficacy of fibrous scaffolds by considering the integration of porosity and interconnectivity of the pores [74]. In other study polyethersulfone (PES) nanofibrous scaffolds were prepared by fine tuning of electrospinning parameters and were evaluated for wound dressing applications. Epithelial regeneration was expeditiously accelerated under PES as compared with Vaseline gauze. Based on results, it was concluded that the biocompatible PES nanofibers can effectively be used as a dressing to accelerate wound healing [77].

5.2.2. Decellularized ECM

This scaffolding approach removes the allogenic or xenogenic cellular antigens from the tissues as but preserves the ECM components. In a study, decellularized

human amniotic membrane (HAM) revealed a well-designed growth pattern of human bladder smooth muscle cells (HBSMCs) with abundant cell-to-cell adhesions [50].

5.2.3. Cell Sheets with Self-Secreted ECM

This scaffolding represents an approach where cells secrete their own ECM upon confluence and are harvested without the use of enzymatic methods. Several researchers examined harvesting of epithelial cell sheet to support for epithelial cell grafting especially cornea regeneration [75]. Biazar and coworkers investigated the effect of $\alpha\gamma$ -pre-irradiation dose of radiation under appropriate temperature and grafting conditions onto a polystyrene cell culture dish. Results indicated that the cells detached spontaneously without using enzymes, and proved that this type of grafted material has potential as a biomaterial for cell sheet engineering [76].

5.2.4. Cell Encapsulation in Polymer Gels

Cell encapsulation is a process entrapping living cells within hydrogel and provides cells with a three-dimensional environment similar to that experienced *in vivo*. Naturally, occurring polysaccharides derived from algae, sodium alginate is the most commonly used material by Iranian researchers [25].

5.2.5. Hybridization of Synthetic/Natural Polymers

Synthetic scaffolds lack good cell interactions. In contrast, natural polymer has the potential advantages of specific cell interactions, but the scaffolds have poor mechanical strength. Therefore, Iranian researchers hybridized natural polymers and inorganic scaffold to combine the advantageous properties of both constituents [17,20,28,37-39,50-52,78,79]. In study of Seyedjafari and coworker, nanohydroxyapatite (n-HA) was coated on poly (L-lactide) (PLLA) nanofibers. The n-HA/PLLA electrospun scaffold showed the capacity for ectopic bone formation in the absence of exogenous cells [20]. In similar study, a nanostructured scaffold was designed for bone repair using hydroxyapatite (HA) and gelatin (GEL) as its main components. Results showed that nano-HA powders can be synthesized and used to make 3-dimensional HA/GEL nanocomposites in any desired shape, with mechanical properties comparable to spongy bone [28]. Also Aboudzadeh and coworkers fabricated scaffolds based on polymer/bioceramic composites. They fabricated and characterized poly (D, L-lactide coglycolide)/hydroxyapatite nanocomposite scaffolds for bone tissue regeneration [51]. Likewise Hajiali and colleagues prepared poly (3-hydroxybutyrate) with bioglass nanoparticles by the salt leaching processing technique, as a nanocomposite scaffold [78]. In other attempts to improve mechanical properties of collagen, Mohajeri and

coworkers prepared a novel porous scaffold for bone tissue engineering with collagen sponge reinforced by polypropylene/polyethylene terephthalate (PP/PET) fibers [38]. In other study a scaffold composed of Poly (epsilon-caprolactone), collagen and polyethersulfone was fabricated by the electrospinning technique which supports and enhances MSC development into functional hepatocyte-like cells [39]. To manufacture a new hybrid sponge scaffold, Eslaminejad and colleagues prepared a composite of beta-tricalcium phosphate-alginate-gelatin. Results indicated that the scaffolds were cell compatible and, more importantly, possess some osteo-inductive properties [79].

6. STIMULATION (MECHANICAL)

Mechanical stimulation plays a critical role in natural tissue development. This stimulus generally applied by bioreactor to composition and ultrastructural distribution of the extracellular matrix and thus the structural integrity of TE constructs. Mechanical stimuli are particularly important for tissues that support mechanical loads *in vivo*, including bone, cartilage, muscle, and vasculature [16,29,80-82]. Therefore, Ghazanfari and coworkers investigated influence of cyclic stretch on proliferation of hMSCs, alignment of actin filaments, and consequent differentiation to SMCs. Results indicated promoted proliferation of hMSCs by cyclic strain, and cyclic stretch regulated smooth muscle alpha-actin, and reoriented actin fibers [79]. Orazizadeh and coworkers to identify whether integrin-associated protein (CD47/IAP), is expressed by chondrocytes and whether it may regulate integrin-dependent mechanotransduction, studied cells in a resting state or following mechanical stimulation at 0.33 Hz. Results indicated that CD47 associated with alpha 5 integrin and regulated responses of human articular chondrocytes to mechanical stimulation [81-83].

7. CHEMICAL FACTORS

A number of growth factors like platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF) and fibroblast growth factor (FGF) explored for their effects on TE [40,61,63,84]. These factors were investigated independently and synergistically, with outcomes dependent on cell type and culture conditions. A study Investigated differentiation factors such as retinoic acid (RA), basic fibroblast growth factor (bFGF) that influence neuronal markers expression *in vitro* from human amniotic epithelial cells. Results showed the capability of AECs to express neural cell markers and this ability is affected by some factors including serum, noggin, bFGF and RA [40]. The study of Ghaedi underscored the utility of growth factor-containing culture surfaces for stem cell differentiation. Bottom-up signaling from HGF-contain-

ing surfaces promoted hepatic differentiation of mesenchymal stem cells [61]. Similar result gained about effect of hepatocyte growth factor (HGF) and fibroblast growth factor (FGF-4) on Mouse BMMSCs [63]. In addition, members of the Bone morphogenetic protein (BMP) family have been shown to play a major role in differentiation process. Results of Makoolati and coworkers suggest that BMP4 is an efficient inducer in primordial germ cell (PGC) derivation from mouse ESC [85].

8. BIOREACTORS

Bioreactor is defined as a means and model systems to generate and maintain a controlled culture environment to support investigations on cell function and tissue development.

8.1. Role of Bioreactors in *ex Vivo* Engineering of 3D Tissues

In general, the functions of a bioreactor are to enhance cell seeding and mass transfer within the culture medium and to provide tissues with hydrodynamic and/or mechanical stimulation [85].

8.1.1. Cell Seeding on 3D Scaffolds

Static cell seeding is by far the most commonly used seeding method. However, this loading of cells onto a scaffold leads to low seeding efficiencies and non-uniform cell distributions within scaffolds. Several studies reported that 3D-scaffold seeding in bioreactors resulted higher seeding efficiencies and more-uniform cell distributions [85].

8.1.2. Increase of Mass Transport

Static culture of tissues in Petri dish leads to construct with hypoxic, necrotic center, surrounded by a rim of viable cells. Therefore, as tissue constructs should have larger dimensions, mass transfer limitations represent one of the greatest engineering challenges. Bioreactors have been used successfully to increase the mass transport of nutrients and oxygen [85].

8.1.3. Mechanical Conditioning

Mechanical conditioning has been employed to induce, accelerate, and/or enhance tissue formation. In addition, the mechanically active environment present in bioreactors mediates the effectiveness of three-dimensional (3D) scaffolds for tissue engineering [85].

In comparison to developed countries, design and validation of a bioreactor research is harder for scientists in developing countries such as Iran. Among many reasons, scientists in developing countries struggle more for provision of proper set-up and research funding. Consequently, Iranian scientists published a limited articles

related to bioreactor in biomedical journals [86-89]. In a study, Packed-bed bioreactor was used by Golmakany and coworkers for continuous production of monoclonal antibody [86]. Amoabediny and coworkers equipped the special shake flasks with oxygen sensors and then developed an unsteady-state model for a biological system in miniaturized bioreactors [89]. In other attempts Amoabediny and coworkers presented a new online monitoring method for the determination of the CO₂ sensitivity of microorganisms in shaken bioreactors. The major advantage of the new methods was the possibility to determine the metabolic activity, regardless of manual sampling [87,88].

9. CONCLUSION

Injury or a disease in growing countries such as Iran has significantly increased in the past few decades that increased need to tissue and organ transplantation. However, low availability of compatible donor's severely limited transplantation of tissues or organs. Thus, biomedical research activities such as TE and new strategy and therapy concepts for repair of diseased and damaged tissue increased in recent years. Among components of TE, Iranian researchers have great chance of providing stem cells due to governmental funding and supports. During the last years, stem cell researchers have found great public interest in Iran. In the scaffold field of TE, Iranian researchers used hybridization of natural polymers and inorganic scaffold to provide optimal scaffolds for TE. In particular, the researchers used hybridization of polymers to adhere, migrate, differentiation and synthesize new bone matrix. Despite its progress for studying stem cell and scaffold, limitations to TE in Iran include property design and validation of bioreactors. Therefore, one of the biggest challenges for TE in Iran is bioreactors researches.

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