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Scaffold technologies: Using a natural platform for stem cell therapy

Yapı iskelesi teknolojileri: Kök hücre terapisi için doğal platformlar

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ABSTRACT

Several contemplated 'scaffold platforms' have been reported recently for the feasible treatment of various health problems and diseases. These engineered scaffold platforms consist of two important factors namely stem/progenitor cells and biomaterial scaffolds designed for the regeneration of many diseased and injured tissues and/or organs. There are many limitations for the application of treatment modalities of end-stage organ failure. Thus, future advance in tissue engineering will depend on improving new scaffolding methods. Currently instead of regenerating whole organ, decellularization techniques used to harvest native extracellular matrix (ECM) scaffold platform are among the most successful techniques. In the light of this information, in this paper we review the current status of scaffold platform technologies; 'decellularization methods' for various tissues and stem cell therapy strategies for tissue engineering.

Keywords: *Stem cell, decellularization, tissue engineering, scaffold, regenerative medicine*

ÖZ

Son yıllarda çeşitli sağlık sorunları ve hastalıklara yönelik olası tedavi yöntemi olarak çeşitli yollarla tasarlanmış yapı iskelesi yüzeyleri rapor edilmiştir. Çeşitli hastalıklar ve doku/organ hasarları için tasarlanmış yapı iskelesi yüzeyleri iki önemli komponentten oluşmaktadır: Kök/progenitor hücreler ve biyolojik yapı iskeleleri. Son evresinde bulunan organ hasarlarının tedavi yollarında çok sayıda kısıtlayıcı etken vardır. Günümüzde tüm organın yenilenmesi yerine, doğal ekstraselüler matriks yapı iskelesi yüzeyi eldesi için kullanılan deselülarizasyon tekniği en başarılı yöntemlerden biridir. Bu bilgiler ışığında, birçok doku için yapı iskelesi yüzey teknolojilerinin (deselülerizasyon yöntemleri) ve doku mühendisliği için kök hücre terapi stratejilerinin son zamanlardaki durumunu bu derleme ile değerlendirdik.

Anahtar kelimeler: *Kök hücre, deselülerizasyon, terapi, doku mühendisliği, yapı iskelesi, rejeneratif tıp*

INTRODUCTION

During the last decade the incidence of end-stage failure has estimatedly increased across the world. This is notably due to growing risk factors and lack of donor organs. The importance of this issue is still being limited with restricted number of treatment approaches. To work up effective and applicable therapies to treat end-stage failure of different tissues is desperately needed.

The goal of tissue engineering is to replace or regenerate injured tissues and/or organs. Hence, the essential components of tissue engineering are stem cells, biomaterials and a proper environment[1]. Tissue engineered scaffold platforms have to possess some properties. They should (i) promote development of novel tissue with specialized functions, (ii) provide a proper platform suitable for forming of new tissues, (iii) allow the interaction between cells and the ECM during normal tissue formation[2].

Stem cells used in regenerative medicine and tissue engineering should also meet some criteria: (i) they should be available in abundant numbers, (ii) they can be harvested using a merest invasive procedure and (iii) they can differentiate along multiple cell lineage (cardiomyocytes, vascular smooth muscle cells, endothelial cells, etc.).

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Although cells and cell sources are equally important, this review focuses on mostly the former and present development researches in tissue engineering of different organs and decellularization native tissue techniques.

Cellular components for tissue engineering

Regeneration attempts of damaged tissues, firstly consists of cell grafting via direct injection into damaged area of tissues. But the efficiency of cell adherence is very low about 90% of the cell suspension is lost after injection procedure. When consider from this point of view only by itself cellular therapy is currently limited and supports the idea of need for other promoter such as scaffolds[3]. Therefore, studies in recent years focus on tissue engineering strategies including biomaterials and cells which can be successfully engrafted into damaged tissues[4]. Hereby the ideal cell source should have some special properties like easy availability, biocompatibility, intense proliferation potential and safety in terms of genetic perspective[3].

There are several key points in cellular therapy. Choice of stem cell sources is one of the most difficult points in tissue engineering. Embryonic stem cells (ESCs) have ethical problems as well as risk of teratoma. Also the major challenging issue is collecting large amounts of unadulterated cells and ensurance of their long term survival. Another stem cell types which are difficult to obtain and culture in vitro, are hematopoietic stem cells, so they are not preferred by researchers. However, mesenchymal stem cells (MSCs) can be isolated from various tissues, adhere quickly to the culture dish forming colonies, so they can be harvested in adequate number (according to derived origin) in a short time. Additionally, MSCs are considered to be powerful cell types for gene targeting therapy and regeneration[3,5]. Most popular cell types in recent times are induced pluripotent stem cells (IPSCs), and reprogrammed autologous somatic cells. These cells are non-immunogenic but have similar properties as ESCs which made them precious material to be used for regeneration. However, isolation of various types of somatic cells like cardiomyocytes is restricted. Moreover IPSCs senescence has not been understood adequately[3]. Instead of directly using all cell types that contain usable tissue, alternative approach is to support and follow the body's own repair process and then implement these cells in a spatial organization. Today, this is achieved by co-culture technique that mentioned in many studies recently[6,7]. Another point is harvesting effective and sufficient number of cells or differentiated cells. Due to tendency of stem cells to spontaneously differentiate into different cell lineages, the efficiency of engraftment is reduced. From this perspective efficacy of the treatment may be improved, if cells could differentiated into intended cell types ideal for the regeneration of the damaged tissue. Also elaboration of the molecular mechanism and signalling pathways that regulate intended cell types will be facilitated when cells can be differentiated in vitro[6].

Scaffolds for tissue engineering

Cell based therapies are among the most successful promising approaches for tissue engineering. But cell therapy itself has so many disadvantages. Therefore the need for crucial factors like biomaterial scaffolds comes to the fore. Biomaterials have important roles as mimicking the natural environment and providing the physical and biological helpers to the attached cells during the in vivo and in vitro cultivation[3]. Cellular adhesion is one of the most undesirable properties for biomaterials. There are many studies in progress about surface modification of biomaterials[8-10]. Furthermore, optimal biomaterials should degrade

Table 1. Proper scaffold platforms properties for tissue engineering.

Biocompatible
Biodegradable (tissue and damaged based)
Support cell adhesion
Non-immunogenic
Non-toxic
Easy obtainable
Controllable porosity
Provide vasculature for oxygen and nutrients delivery
Providing microenvironment and promote cells growth
Possesses proper biomechanical strength

without toxicity and must control degradation rate[11]. Another important factor of biomaterials is the pore size and pore connectivity which are key mediators of cell behaviour[11,12]. All together, ideal scaffolds properties for tissue engineering are shown in Table 1. Number of different synthetic and natural source have been used as scaffolds for various damaged tissue models[4].

Synthetic scaffolds for tissue engineering

A functional scaffold for tissue engineering should have some mechanical and topographical properties[13]. Synthetic polymers like polyglycolic acid (PGA), poly-L-lactic acid (PLLA) and polylactic acid (PLA) could supply covetable mechanical properties and topographies providing guidance to construction. But because of the elasticity of some tissues, researchers have focused on hydrogel synthetic polymers[4]. In agreement with these results, more elastic synthetic materials namely "elastomers" have come into current issue. Elastomers are known as natural rubber due to their mimicking potential of the flexible tissues. Polyurethane (PU), 1,3 trimethylene carbonate, PEG and poly (glycerol sebacate) (PGS) were reported as examples of elastomers[14-17]. Since large-scale animal studies have not been performed yet, co-decisions about these materials are not fully reliable.

Natural scaffolds for tissue engineering

Several research groups are currently studying scaffold materials composed of natural supplies. There are many different natural scaffolds available. Nevertheless, all types of natural scaffolds have the same properties, in other words they are being biodegradable and biocompatible.

Collagen is the most preferable natural scaffold in tissue engineering research areas, based on its many properties. Because of its biocompatible, biodegradable, adhesive and porous features it can integrate with different natural and synthetic scaffolds. Also its structural speaciality like preserving tissue entirety and promoting the distinctive characteristics of ECM microenvironments made it appropriate for implementation[18-24].

Fibrin is an essential protein which is the active form of fibrinogen. It is produced after inflammation and with the cooperation of macrophages. Its main role is to participate in blood clot formation and wound healing processes[25-27]. When inflammation occurs in the body, fibrin plays important roles in cell-ECM interactions with other matrix components like collagen[25]. Also fibrin has important competency in the required site, and assists cell for biological adhesion[28]. After injury, fibrin derived from blood supports preventive properties for immune rejection. Moreover, fibrin can be manipulated with ease by re-organizing fibrinogen concentrations for alteration of matrix density, microstructure and mechanical strength[29-31]. In consequence of these specifications, fibrin becomes a delivery vehicle and bioactive scaffold in tissue engineering.

Elastic fibers compose of elastin and microfibers, present in all tissues. Elastogenesis is the procedure called for polymerization of water-soluble tropoelastin to form elastin proteins. Additionally, elastin materials are soluble and biodegradable just like other natural biomaterials[32].

Chitosan, a natural derivative of the alkaline deacetylation of chitin, which has been widely investigated for cartilage, bone, liver, skin, blood vessel tissue replacement[33,34]. This material has the capacity to combine with conductive materials[35,36]. On the other hand, chitosan has been shown to have biological properties like being soluble, reactive and biodegradable[37].

Alginate, gel particles, are among the more commonly used natural materials due to their being biocompatible, non-thrombogenic, non-toxic, mild, biodegradable, cheap, and simple to produce. Alginate is an anionic linear polysaccharide which after ionic crosslinking with divalent cations such as Ca2, forms a hydrogel[38,39]. The properties that enables

combination of cells and proteins inside the hydrogel made alginate more useful scaffold type for tissue regeneration[40,41]. Several composites such as alginate-polymer, alginate-protein, alginate-ceramic, alginate-bioglass, alginate-biosilica, alginate and RGD peptides composite have been investigated up to now. Interestingly, recently several studies have demonstrated that it caused reverse remodeling and also after implantation pure alginate did not result in host immune response[42,43].

Hyaluronic acid plays important roles in cell attachment, development of tumor, wound healing, joining of connective tissue, and inflammatory reaction[44]. Hyaluronic acid is largely used in many clinical conditions due to its anti-inflammatory and angiogenic properties. Nowadays this material has been used in different tissue engineering fields. Bone and cartilage restoration, myocardial regeneration, wound repair and nerve-brain regeneration are some of the application areas of the hyaluronic acid[45].

Matrigel is a biomaterial produced from ECM and its composition is still not clear. But its main property is that it can support angiogenesis both in vitro and in vivo[46]. This characteristic properties made it useful platforms for cellular therapy.

Gelatin is produced as a result of induction of partial hydrolysis of bone, skin, or tendon collagen. Known as a natural polymer, gelatin is an ideal material for tissue engineering applications due to its many properties. It is biocompatible and biodegradable and also has low antigenicity. Another important feature is that it is cheap[47,48].

ECM is the perfect combination of the proteins and some important molecules of tissue and organ origin[49]. This physiologic structure make cells suitable for their proper circle systems including different signals for proliferation, differentiation, migration and attachment[50,51]. The cells and their contact material ECM are in a state of "dynamic reciprocity", which means that they are really in communication with a good balance[52]. Decellularization procedure, including removing complete cellular and nuclear components, is the way of ECM extraction[53,54]. Successful decellularization technique requires correct selection of physical, chemical, and biological procedures that can remove cellular antigens without damaging ECM. Indeed, cell-free ECM must maintain its mechanical integrity and biological activity. Altogether, decellularized ECM scaffolds can serve as "constructive remodeling" of damaged tissues[55].

Decellularization methods

Based on various studies, several different chemical, biological and physical procedures and their combinations have been developed to remove both cellular and nuclear components with minimal disruption of ECM. These various detergent-based techniques end up with decellularized tissue differing in ECM composition[56]. Correspondingly, composition of decellularized scaffolds used in different protocols will influence the cell seeding and attachment potentials. This is the main point that scientists are now focusing on the selection of decellularization protocol and develop most potential clinical usability of decellularized tissue scaffolds.

Decellularization method primarily aims to lyse membrane cells using physical methods or ionic solutions. Then, for separation of cellular and nuclear components from ECM, enzymatic methods are provided. Detergents are used to dissolve the nuclear and cytoplasmic cellular components. Finally, it aims

Table 2. Decellularization methods used in general.

Chemical	Biological	Physical
*Alkaline-acid treatment *Non-ionic detergents *Ionic detergents *Zwitterionic detergents *Tri(n-buyl) phosphate *Hypotonic and hypertonic treatments *Chelating agents	*Protease inhibitors *Calcium chelating agents *Nucleases *Antibiotics	*Freezing&Thawing *Mechanical force *Sonication *Mechanical agitation

to completely free the tissue from cellular debris. These steps can be supported to increase their effectiveness by mechanical agitation method. Commonly used decellularization methods are briefly shown in Table 2.

Heart

End-organ heart failure is increasing day by day because of the lack of donors and immune rejection problem which promoted the development of tissue engineering and regeneration medicine. Midlevel milestone about replacement or regeneration of injured heart is the restoration of the cardiac functions completely which has not been succeeded to date. Hence, many scientists have made experimental studies on various engineered tissue platforms for the regeneration of the infarcted heart tissue. These engineered tissue platforms base on cells and biomaterials especially for the regeneration of the infarcted heart tissue. In the first instance polylactic acid, polyglicolic acid and polylactic-co-glycolic acid- based biodegradable and biocompatible polymers used for the repair of the damaged heart[57]. Then, elasticity problem encountered and most research focused on hydrogels composed of natural and synthetic polymers and their composites. Initial natural polymer is collagen, which is the major component of ECM. Some studies have showed that collagen strings improved cell morphology and support contractility function[58-61]. It was also demonstrated that collagen patches provide cell viability up to eight weeks after implantation[60]. Another research group reported that they produced a complex and biocompatible ECM scaffolds with perfusion decellularization[62]. Same group declared that they applied same protocol to big mammalians (like pig) and showed that this technique can be scaled to human heart size and complexity. Similarly, in an another research group, ECM scaffolds obtained by using the combination of decellularization methods (physical, chemical and biological) showed the same mechanical properties of natural myocardium[63]. Additionally, another study proposed that ECM patches would not only need to be elastic-compatible but also be thick and perfused with ease for cell viability[64].

Recently, patch platforms are the most popular biomaterials which have been studied by various research groups especially for cardiac regeneration. It can be divided into two main groups as cell sheets and synthetic and natural polymers with or without cell combinations[3]. Up to the present, for cell sheet transplantation many types of cells including skeletal myoblasts, adipose tissue derived MSCs, neonatal rat hearts cardiomyocytes and endothelial cells, adipose-derived stromal cells with ESCs derived cardiac progenitor cells and IPSCs were applied[65]. Synthetic and natural polymers were described in the previous sections.

Most recently Russo et al.[12] reported new method for decellularized left ventricular myocardium namely 3D porous foams which are stable in culture and obtained without chemical cross-linking. Contrast to other studies, this group synthesized the cardiac ECM gels and foams with pepsin digestion and polymerized collagen with α-amylase digestion.

Lung

The goal of tissue engineering and regenerative medicine is to restore or replace damaged parts of tissue or body. Recently, these two disciplines have allowed obtaining functional tissues in in vitro. Blood vessels, urinary bladder and trachea can be examples of these tissues. Most important differences are that these organs don't need a large vascular network. However, the larger tissues like lungs, heart, and liver urgently require blood supply. Especially, bioartificial lung requires gas exchanging, perfusion and oxygen ventilation.

Based on various studies, they demonstrated that vascular and airway components, as well as pulmonary matrix, can be decellularized with different chemical agents and zwitterionic detergents[1]. All groups that are mentioned in this section, showed the intact structure of major components of ECMs. Lemon et al.[66] demonstrated cell sources and types of bioma-

terials for the bioartificial lung with their advantages and disadvantages. The major cell types suggested by them are epithelial cells (EPCs) and endothelial cells (ECs). Alveolar macrophages and smooth muscle cells are the other cell types of natural lung. Moreover, they recommended and discussed the potential of different kinds of sources for these cells.

Liver

Currently, obtaining bioartificial liver is a bit complicated. In the earlier studies ex vivo systems with synthetic matrix had been described as a promising approach for replacement liver transplantations[67,68]. Therefore, several research groups focused on perfusion decellularization technique for producing 3D scaffold platform for damaged liver[69-72]. In summary, groups tested different perfusion protocols with different time durations for various animal livers in different size and structure. All groups showed that hepatocyte seeded decellularized liver platforms functional at implanted side.

Matsuura et al.[65] indicated the importance of hepatocyte transplantation due to their major roles in in vivo settings like suppressing toxins and supplementing different substances. The same study demonstrated that cell sheet technologies in compliance with gene transfer techniques support function of liver affected with metabolic disorders in in vitro and also at ectopic site of animals. They constructed pre-vascularization devices including basic fibroblast growth factor to prolong the survival of transplanted hepatocytes. Another research group suggested Human Wharton's jelly MSCs (HWJMSCs) for cell replacement therapy for liver disorders[73]. HWJMSCs have a large gene expression profile. From the point of liver regeneration, HWJMSCs express the early hepatic markers. Khodabandeh et al. demonstrated that the HWJMSCs showed different behaviours in 2D and 3D culture systems. 3D collagen scaffold platforms promote HWJMSCs to express tight junction markers, especially claudin. Besides, hepatic nuclear factor-4 expression is stimulated on the 2D collagen scaffold films[73].

CONCLUSION

Consequently, due to high prevalence of organ failures and limited success with traditional medical and surgical therapies, the replacement of damaged tissue remains an urgent and difficult task. Under these circumstances, different tissue engineering targets at assembling tissue constructs that can restore basic functions of various tissue by combining cellular components with scaffold platforms which in turn provide a framework of ideal structural, mechanical, and electrophysiological characteristics have been investigated. Recently, much emphasis has been placed on tissue engineering methods that mimic the biological and biomechanical components of the native tissue and maintain transplanted cell function and survival[74]. The three main targets are biomimetic decellularized scaffold platforms, proper cell types and microenviroment. Mechanical interaction between cells and the scaffolds plays an important role in the morphogenesis and function of tissues. Therefore, the use of decellularized matrices within various tissue-derived stem cells or IPSCs has the most potential approach for overcoming the need for bioartificial tissue engineering.

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