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FULL LENGTH REVIEW

The useful agent to have an ideal biological scaffold

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Abstract Tissue engineering which is applied in regenerative medicine has three basic components: cells, scaffolds and growth factors. This multidisciplinary field can regulate cell behaviors in different conditions using scaffolds and growth factors. Scaffolds perform this regulation with their structural, mechanical, functional and bioinductive properties and growth factors by attaching to and activating their receptors in cells. There are various types of biological extracellular matrix (ECM) and polymeric scaffolds in tissue engineering. Recently, many researchers have turned to using biological ECM rather than polymeric scaffolds because of its safety and growth factors. Therefore, selection the right scaffold with the best properties tailored to clinical use is an ideal way to regulate cell behaviors in order to repair or improve damaged tissue functions in regenerative medicine. In this review we first divided properties of biological scaffold into intrinsic and extrinsic elements and then explain the components of each element. Finally, the types of scaffold storage methods and their advantages and disadvantages are examined.

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Introduction

Tissue engineering has appeared in the 1980s. This multidisciplinary field is applied in regenerative medicine to help various damaged tissues and organs, and it is based on using of cells, scaffolds, and bioactive factors. Scaffolds not only provide a supportive template for cell attachment, but they also create a biomechanical and physical environment. So the scaffolds play an active role in the regulation of cell behaviors (Qiu 2012).

Because of the toxic and inflammatory capacity of synthetic polymers, which lead to reducing extracellular matrix (ECM) remodeling and growth capacity, the xeno-or allogeneic tissues are substituted to biodegradable synthetic scaffolds (Thompson 1992). The cells of xeno-or allogeneic tissues as biological scaffolds, are removed, and their ECM remains as 3-dimensioal (3D) structure (Badylak et al. 2009). These natural ECMs decrease immune and inflammatory response in grafting through decellularization, and serve as inductive means through their structural and functional proteins and endogenous growth factors (Assmann 2013; Badylak et al. 2012).

Collagen, elastin, and various glycosaminoglycans (GAGs), are the main component of biological scaffolds, in which collagen needs to form stable structures, then elastin provides elasticity and flexibility of ECM, and eventually, GAGs cause adhesion, migration, proliferation, and differentiation of cells (Jackson et al. (1991); Stringer and Gallagher 1997). Also, bioactive factors that are preserved in biological scaffolds, have an essential role in regulatory signals and functions (Crapo et al. 2011; Gilbert et al. 2006).

During the decellularization process and preservation of biological scaffolds, the amount of these elements, especially bioactive factors, may be diminished or led to the inactivation of bioactive factors (Crapo et al. 2011; Gilbert et al. 2006). So using exogenous bioactive factors can improve this deficiency. Systematical and local application of exogenous bioactive factors is not suitable options because of the following reasons, including fast diffusion of factors in body fluids, which may create unsatisfactory side effects; rapid clearance of factors from application site and low half-life of them in circulation, which required repeated doses and caused raising remedy cost (Nagase 2007; Moreno 2005; Liu 1994; Ohno 2007). So the researchers come to the point that the loading of exogenous bioactive factors into various scaffolds would be an alternative method (Singh et al. 2008).

In this review, we study the useful agents to have an ideal biological scaffold. One of these agents is how to prepare the scaffold, which can be divided into two categories: intrinsic and extrinsic elements. Intrinsic elements including the condition of factors which belong to the ECM itself and should/not should be preserved, and, extrinsic elements consist of various biological and non-biological components that do not belong to the ECM and must be added to or removed from it Fig. 1. Another agent is how to store the scaffold, which includes various methods of short-term and long-term storages with their own advantages and disadvantages.

Intrinsic elements

Immunological status

Decellularization process aims is to produce acellular tissue, which has following properties: (1) without any remnants of the cellular component such as cell membrane, nucleic acids, and mitochondria; (2) without any immunological elements; (3) without any cytotoxic elements, and, (4) without any part which triggers calcification process. Considering the maintenance maximum natural state of ECM, it should be emphasized that any decellularization methods or combination of them do not remove 100% of cellular components from tissues (Kawecki 2018). As a result, the sufficient removal of cellular components from xenograft tissues is vital to avoid an undesirable immune response (Kim et al. 2002).

Before proceeding to discuss the sufficient removal of cellular components from xenograft tissues, let us characterize the difference between the host remodeling and the host immunological reactions: degradation of matrix proteins within xenogeneic scaffolds after implantation, without any adverse immune reactions, is needed for tissue reconstruction. But the deterioration must be at an appropriate rate, fast enough to minimize the possible unwanted immune response, and yet slow enough to retain the host matrix remodeling process (Kim et al. 2002).

The materials of scaffolds cause changes in the population of macrophages immediately upon implantation. These changes include the conversion of M1 macrophages (pro-inflammatory and cytotoxic agents), to M2 macrophages (anti-inflammatory and pro-healing agents), and stimulate Th2 lymphocytes which inhibit macrophages activation and generally contribute in transplant acceptance (Brown 2009, 2012; Badylak 2008; Allman 2001, 2002). Eventually, these immune response cascades promote degradation and the constructive remodeling of scaffolds.

So anti-inflammatory responses are associated with host remodeling reaction, but pro-inflammatory responses, which cause encapsulation and foreign body rejection, are related to adverse immunological responses (Daly 2012). Finally, it must be said that the change of degradation rate can alter the host response. The rate of degradation, in turn, can be changed by different tissues and diverse tissue sources, which include various quantities of immunological components, and the age of tissue sources, which causes a change in the composition of ECM (Record (2001); Carey 2014; Tottey 2011).

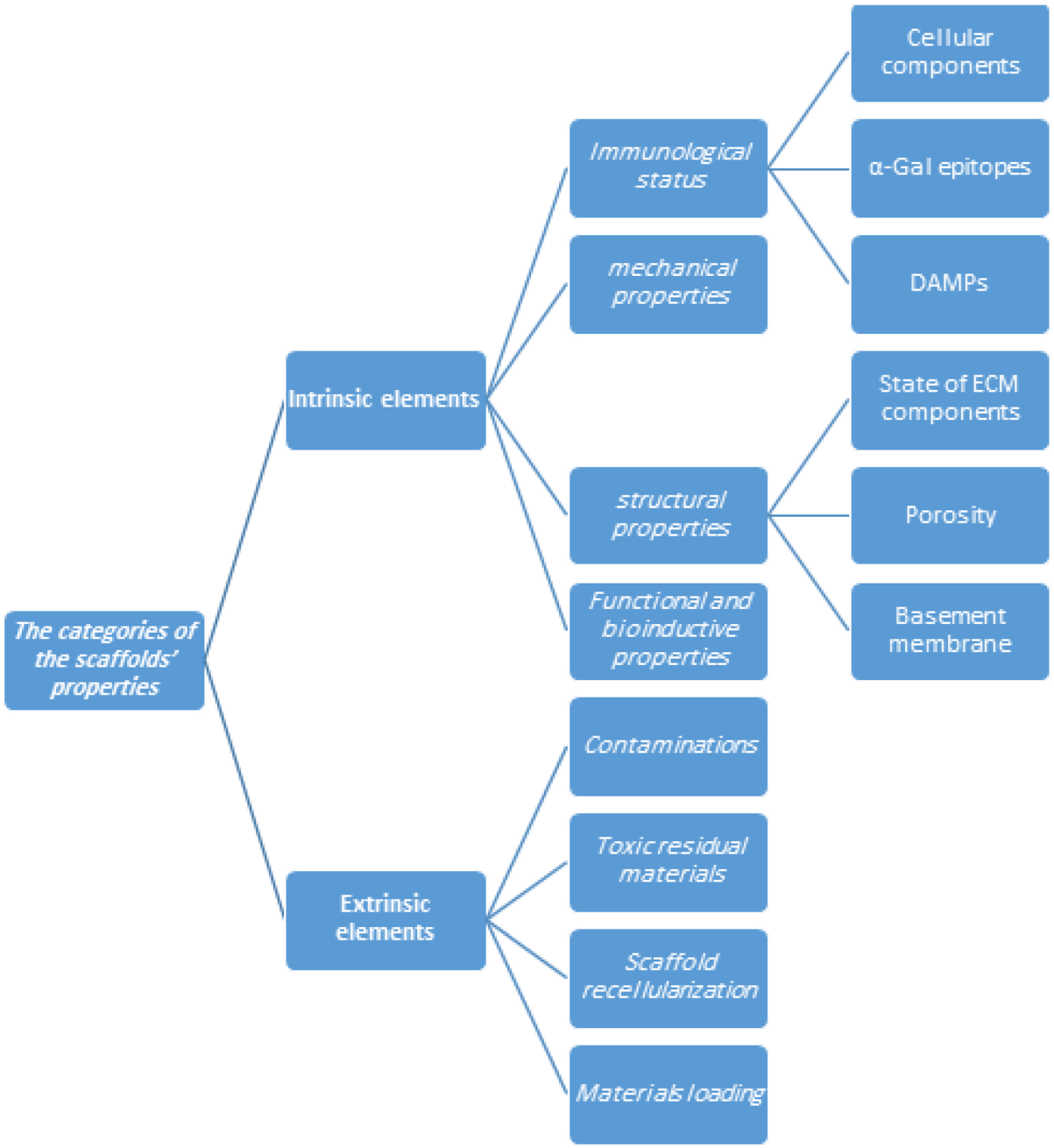

Fig. 1 The categories of scaffolds' properties

Cellular components

Based on the findings obtained from in vivo studies, in which constructive remodeling response was observed without host immunological response, the following criteria are proposed to ensure decellularization methods (Crapo et al. 2011): First, the absence of intact cells and their nucleus are examined. In the next step, the amount and the size of DNA fragments are measured, and they should be less than 50 ng and 200 pb, respectively.

It should be considered that the remnants of cellular debris, including hydrophilic and lipophilic antigens

in allogeneic or xenogeneic scaffolds, may promote the rejection process upon implantation (Wong 2016). The dominant histocompatibility complex class I (MHC-I) or human leukocyte antigens I (HLA-I) molecules in humans, which are presented on the surface of almost all nucleated cells, especially are considered to be xenoantigens or alloantigenics respectively, and initiate the immune response and inflammation (Cascalho and Platt 2001; Yang and Sykes 2007). So the usage of lipophile solubilization and hydrophile solubilization steps during the decellularization process are a useful strategy for avoiding fibrous encapsulation and immune rejection (Wong 2016). Eventually, it should be noted that the remaining nucleic acids may trigger the calcification process, so the extraction of residual DNA and RNA should be considered (Poornejad 2016).

α-Gal epitopes

Galactose-α-(1,3)-galactose terminal carbohydrate epitopes (α-Gal) exist in the tissues of all mammals and most lower creatures except in old world primates and humans, so in xenograft implantation, in primates and humans, these epitopes should be considered (Sandrin 1993). The lack of α-Gal epitopes in primates and humans may result in a high level of α-Gal antibody in the circulation during transplants, and in turn, create a significant inflammatory response or a hyperactive rejection response to xenograft scaffolds (Xu 2009).

Several methods have been used to eliminate these epitopes, and diminish the rejection response. These methods include the following: transgenic modification, structural masking and enzymatic removal of xenogeneic epitopes (Xu 2009; Galili et al. 1997; Stone et al. 1997). However, in commercial ECM scaffolds, the remaining of α-Gal epitopes have been found and have not been had any lousy effect during in vivo ECM remodeling (Daly 2009; Raeder 2002). Similar to the remaining DNA fragments, although the residual α-Gal epitopes should be stimulated an immune rejection, it is likely that a threshold amount is likely needed to create adverse effect on the ECM remodeling response (Badylak and Gilbert 2008). So the low amount and highly scattered distribution of α-Gal epitopes cannot activate immune response (Raeder 2002). Finally, it should be noted that none-α-Gal epitopes have been seen, still produce immunogenicity (Chen 2005; Lam 2004).

DAMPs

Damage associated molecular patterns (DAMPs), or alarmins are multifunctional proteins. They exist in the nucleus, cytoplasm or exosomes, preserve intracellular homeostasis, and have no secretion signals typically, but they can be secreted by macrophages or released by necrotic cells and act as endogenous danger signals to the immune system (Srikrishna and Freeze 2009). However, they function as pro-inflammatory, chemotactic, proliferative, and tissue regeneration agents (Daly 2012). Heat shock proteins, high mobility group box1 (HMGB1), S100 proteins, hyaluronan and heparin sulfate belong to the DAMPs, which are known to date (Rubartelli and Lotze (2007)). Between them, HMGB1 is the best characterized of the DAMPs and acts as a DNA binding nuclear protein intracellularly (Thomas and Travers 2001). As already mentioned, complete removal of cellular components and DNA is not achieved by a variety of decelullarization methods, so that DAMPs maybe existed in biological scaffolds.

The DAMPs do not just create a negative host response upon implantation, but their effects are more complex than this. For example, in addition to inducing the release of pro-inflammatory cytokines and chemokines by HMGB1 in some disease conditions, it can also be a chemotactic and, or proliferative agent for some cell types (Lolmede 2009; Ranzato 2009). These results may be due to the binding of HMGB1 to a variety of molecules and receptors, which in turn activate various intracellular signaling pathways. On the other hand, this should be taken into account that the content of HMGB1 within scaffolds depends on tissue source, decellularization protocol, and the crosslinking agents, which are used during scaffold processing steps (Badylak 2014).

Structural properties

Microscopic and ultrastructural features of the scaffold have an essential role in the regulation of cell behaviors such as the ability of cell migration into the scaffold (Brown 2006a) or determination of cell phenotype (Gong 2008; Sellaro 2007). So the preservation of the ultrastructure and 3D architecture of the

scaffold is vital throughout processing steps of the tissues during decellularization (Brown 2006a; Sacks and Gloeckner 1999). But it should be noted that every the decellularization method will alter ECM components and create some disruption in its ultrastructure. Therefore, one of the goals that should be considered in decellularization process is to minimize these unwanted results (Crapo et al. 2011).

State of ECM components

The maintenance of the state of major ECM components, including the natural structure, regular arrangement, and distribution of collagen fibers without any obvious muss or tear, leads to the preservation of the scaffold ultrastructure (Rashtbar 2018). Such conditions can provide unique orientation, which will help in vitro or in vivo recellularization (Scarritt et al. 2015). On the other hand, in some acellular tissues involved in accommodation such as blood vessels, bladder or skin, the evaluation of elasticity is also done (Amiel 2006; Song 2014; Debels 2015). In addition to collagen and elastin fibers, the maintenance of the microvasculature and capillary bed will be a curtail feature to successful recellularization (Rashtbar 2018; Scarritt et al. 2015).

Porosity

Since dense structure can inhibit the ingrowth of host tissue and neovascularization (Lee et al. 2015), a certain degree of porosity of scaffold has an important role in cell infiltration and proliferation (Cartwright 2006). The pores of scaffold create a larger surface area for exchanging of nutrients and metabolic waste, and make better the mechanical interlocking between the scaffold and the surrounding tissue and may facilitate the integration of them in implantation (Lee et al. 2015; Karageorgiou and Kaplan 2005). Interconnections among pores facilitate cell migration into internal pores, favorable transport of nutrition and waste, and increase cell communication in different pores (Yang 2008). Finally, scaffolds with appropriate porosity and suitable pore size are ideal for loading drugs and factors (Yan 2018).

Water absorption ability or swelling ratio of scaffold is one of the critical factors, which is affected by porosity and usually evaluated along with it (Ma 2004; Jiang 2013). The scaffold with highly porous structure can retain a large amount of water within itself, which in turn hold the nutrients and transfer the metabolites to accelerate cellular infiltration, adhesion, growth, and proliferation (Mao 2003; Zhang 2011).

Basement membrane

The basement membrane is a dense part of the ECM and prevents cell migration into the underlying connective tissue (Brown 2006a). This ultrastructure is in contrast to the underlying matrix, which has irregular fibrous architecture, and facilitates cellular mobility and penetration of cells into the scaffold (Brown and Badylak 2014). So if invasive growth of cells into the scaffold is required, the scaffold with meshwork surface should be used. Alternatively, if noninvasive growth of cells is needed, such as epithelial cells, the scaffold with an intact basement membrane may be more practical (Brown 2006a).

Therefore in the study of some acellular tissues, mainly derived from hollow organs (such as blood vessels or bladder), evaluation of luminal and abluminal side features is also done (Amiel 2006; Coakley 2015). The smooth, dense surface of the luminal layer shows the status of the basement membrane, and a network of collagen fibers of the abluminal side demonstrates the porosity of this layer (Coakley 2015)..

Mechanical properties

The mechanical properties of scaffolds are directly affected by the components of the tissue, such as collagen fiber, GAGs, and elastin, and how they are arranged within ECM (Du 2011). In modulation of many cellular functions such as proliferation and alignment of cells, ECM components expression and biomechanical properties of tissue, the mechanical forces are critical (Wang and Thampatty 2006; Grenier 2005). So the mechanical properties of the scaffold should be similar to those of the tissue at the implantation site, or sufficient to supporting and resisting against the surrounding pressure without inhibiting suitable biomechanical conditions (Garg 2012).

Shortly after implantation, the strength of scaffold typically decreases, which is temporally associated with degradation of the scaffold in the defect site.

However, after residing in the infiltrating cells, the new ECM produces and rapid scaffold remodeling occurs, which in turn increases the strength and mechanical behavior until the normal function of tissue has been restored (Badylak 2001,2005). So the mechanical behavior of scaffold alters during the remodeling process, and such changes are affected by the rate of the scaffold degradation, and the speed and extent to which the infiltrating cells deposit new ECM (Badylak et al. 2009; Badylak 2001). Therefore, as already mentioned, the optimal speed of degradation of scaffold, in addition to adverse immunological reactions, plays a role in its mechanical properties. So that rapid degradation or absorption of scaffold along with unbalanced new ECM production, results in the formation of fibrotic scar tissue in the reparative process (Xu 2009).

Also, according to the elastic modulus of matrices, scaffolds mimicking the brain, muscles or bone, were neurogenic, myogenic, and osteogenic, respectively (Engler 2006). So the mechanical environment, which is sensed by seeded cells, can affect the differentiation of stem cells and the recellularization (Agmon and Christman 2016). On the other hand, in some biological condition such as filling and emptying of the bladder the biomechanical factor is so critical (Boruch 2010); or since progressive weight-bearing and early rehabilitation in tendon and ligament repair, the mechanical properties of scaffolds should be superior to the host tissue (Chen 2009).

During the preparation of acellular scaffold, a concern with this process is the disruption of collagen and elastic structure in the scaffold and some removal of GAGs from it, which will decrease the mechanical strength and viscoelasticity, and increase the biodegradation rate of the scaffold (Kawecki 2018). So the ideal scaffold should have enough mechanical properties to be appropriate for the surgical application, and maintain its original strength and surface area during remodeling process to prevent failure, shrinkage, bulge, or stretch (Hammond 2008).

Considering that the alignment and organization of collagen fibers are related to the function of the source tissue, an understanding of these characterizations of the collagen fiber is essential for the design of scaffolds. Besides, the mechanical behavior of single or multilayer of ECM is important for load-bearing application in clinical use (Badylak 2007). Finally, it should be noted that apart from the decellularization process, there are other factors e.g., the age of the animal, diet, or race, which influence the mechanical strength of the scaffold (Rashtbar 2018).

Functional and bioinductive properties

In addition, to create structural integrity, the maximum preservation of the ECM component during the decellularization process, can provide necessary spatial and contextual signals for various behaviors of cells and the production of varied secreted mediators (Booth 2012). So these components can turn cell-free scaffolds into a niche that recruits stem or progenitor cells and assist them in differentiating into functional tissue (Kawecki 2018). The ECM is an extremely dynamic structure that is continuously being replaced, revised and restored, and varies in composition according to the local and physiological situation of tissue (Booth 2012; Boudreau et al. 1995; Ingber 1991).

Based on the tissue from which the scaffold is derived, different types of collagens, glycoproteins, sulfated GAGs, and bioactive factors exist in the ECM (Yang 2010). Collagens are the most abundant component in the ECM, and because of their Arg-Gly-Asp (RGD) sequence, that is a usual ligand for different integrins, they play an essential role in cell adhesion (Hynes 1992; Kanematsu 2004; Khoshnoodi et al. 2008).

Two of the most important glycoproteins of ECM are fibronectin and laminin. Fibronectin is abundant in the RGD, which is essential for cell adhesion (Badylak 2004a,2002; Hirschi et al. 2002). Also, it plays a role in growth, migration, and differentiation of cells (Schwarzbauer 1991; Miyamoto 1998). It can bind other proteins such as collagen and act as a chemoattractant for fibroblast (Hirschi et al. 2002; Thibeault et al. 2003). Laminin also plays a role in cell adhesion through its YIGSR and IKVAV polypeptide sequences (Arenas-Herrera 2013).

GAGs usually are found in the form of proteoglycans in the ECM. They can bind to various proteins of ECM and modulate their functions (Stringer and Gallagher 1997). One of the most essential roles of GAGs is the protection of free growth factors from degradation (Saksela 1988). Another purpose of them is of importance in morphogenesis, which is vital for the recellularization process. The last part includes the maintenance of original phenotypes of repopulated

cells, induction of cytoskeletal rearrangement, and cell shape changes and motility (Brown 2006b). Finally, they promote the retention of water and control the hydration of the ECM, so they are vital to the maintenance of intermolecular spacing for cell migration (Badylak 2004b).

After cell seeding and in vivo implantation of a scaffold, the behavior of cells, and the remodeling process will be affected by growth factors, and bioactive molecules (Boruch 2010; Arenas-Herrera 2013; Badylak 1995,2011). These effects occur during scaffold the degradation. The growth factors such as VEGF, bFGF and TGF-β are dissociated from GAGs, activated and exert their biological effects (Voytik-Harbin 1997; Hodde 2001; McDevitt et al. 2003). On the other hand, during degradation of the parent molecule, such as collagen and fibronectin, some fragments are produced. These products or cryptic peptides (such as endostatin derived from collagen XVIII) mediate a series of biological activities such as angiogenesis, anti-angiogenesis, antimicrobial and chemotactic effects (Kawecki 2018; Brennan 2006; Li et al. 2004; Zantop 2006). Finally, it has to be said that the age, in addition to structural changes, also influences functional changes in scaffold; so that this factor affects extracellular matrix composition and in turn causes behavioral changes in cells that are placed on the scaffold (Godin 2016; Smith 2017).

Extrinsic elements

Contaminations

Given that the scaffolds themselves have antibacterial activity (Sarikaya 2002), sterilization is an essential procedure in the preparation of biological scaffold. The purpose of this step is to minimize unwanted immune response by the elimination of any endotoxins and intact viral and bacterial DNA (Crapo et al. 2011), while preserving the structural, mechanical and biological properties of the scaffold (Kajbafzadeh 2013). Although some studies have shown that the aseptic process can remove the vast majority of microorganisms from the scaffold (Mendenhall 2017), the ideal decellularization process might not create enough sterilization (Song and Ott 2011). Even the use of antibiotics and antimycotics for disinfection of scaffold has low decontamination efficacy, because a percentage of donated scaffold has been rejected as a result of contamination (By 2012). Finally, keep in mind that unlike allogeneic tissues, xenogenic ECM scaffolds are classified as medical devices (Nichols et al. 2012); and because of endogenous viral-associated risk (Knight 2008), they require a validated sterilization technique for the competent national and international authorities such as Food and Drug Administration (FDA) in US or Good Manufacturing Practices (GMP) in EU (Nichols et al. 2012).

Therefore, before implantation or in vitro use of biological scaffold, we required a sterilization method that while safe for scaffold, also provides good antiseptic results (Hussein 2016). For new methods of sterilization, a careful examination is needed to evaluate the effectiveness of the protocols.

Toxic residual materials

An ideal scaffold should possess acceptable cytocompatibility for adhesion, migration, and outgrowth of various cells (Zvarova 2016). During the preparation of the ECM scaffold, several factors are involved, which can affect biocompatibility and toxicity profiles. These factors include the type and concentration of reagent used for decellularization process (Wang 2015; Fermor 2015), the time of scaffold exposure to the reagent (Sullivan 2012), the pH at which reagent is used (Yang 2010), the duration of washing step (Starnecker 2018), and the use of chemical material for crosslinking and sterilization (Badylak et al. 2009). Even different cells exhibit different cytotoxic thresholds to various reagents (Zvarova 2016). So there is a need to assess the presence of any toxic residual materials in the ECM scaffold. For this purpose, there are two methods: extract cytotoxicity assay and contact cytotoxicity assay. In the latter method, in addition to cell viability, the morphology of cells is also examined (Wilshaw 2006). In addition to cell-based cytocompatibility assessment, there are other techniques that can detect the presence of residual reagents in the scaffold (Zvarova 2016).

In addition, to reduce antigenicity and increase mechanical properties (Xu et al. 2007), the chemical crosslinking materials can influence the degradability, and therefore, the host response to the scaffold (Badylak et al. 2009). In other hands, some of reagents that are used for the decellularization process (e.g., DNase, RNase, or trypsin), generally derived from

bovine sources. These enzymes may potentially create an unwanted immune response once implanted (Gilbert et al. 2006). So the in vivo investigation also will be required. It should be noted that the evaluation of the safety of extractable and leachable substances is extremely important for authorities such as the FDA to medical device submission (Jenke 2007).

Scaffold recellularization

Recellularization of scaffold is necessary for successful tissue regeneration. This process can be done in two manners: 1) in vivo implantation acellular the scaffold and usage of host cells post-surgery, 2) transplantation of cell-seeded scaffold ex vivo (Wilson 2013); these cells can include autologous, allogeneic and xenogeneic. Recellularization of the scaffold has several benefits, such as preventing thrombosis after endothelialization (Kasimir 2005), allowing ECM to undergo turnover, repair, adaptive remodeling, and growth (Park 2009; Nam 2012), gene therapy and have biological functions which mediated by special cell (Borschel et al. 2004).

In addition to mesenchymal stem cells, the differentiated cells such as epithelial, stromal and endothelial cells are also used in ex vivo recellularization. Apart from the cell source, cell density and method of cell delivery also vary in this process (Wilson 2013). The cell density should be chosen to keep cells from contact inhibition and cell aggregation, but also create a confluent cell layer on or throughout the scaffold (Scarritt et al. 2015; Proulx, S.p, et al. 2009). Considering the basement membrane, the method of cell delivery can be seeding or injection. As already mentioned the basement membrane prevents cell migration into the underlying connective tissue (Brown 2006a). So even in vivo epithelialization can be done rapidly without complication, but for stromal cells intra-stromal injection ex vivo has shown better results (Wilson 2013).

In the case of the contractile cells, it should be borne in mind that the contraction effect can reduce the pore size of scaffold, which in turn influences the cell proliferation and diffusion of the nutrition and waste (Ma 2004). So the cell-mediated contraction (CMC) of the scaffold must be measured. Finally, considering the limitation of oxygen diffusion, which is 150–200 μm in the human body (Scarritt et al. 2015), the thickness of non-vasculature scaffold is a critical feature in successful repopulation (Walles 2003). To determine success of scaffold recellularization histological techniques including light and electron microscopic assay, cell proliferation and cytotoxicity assay are used. Typical methods for cell seeding on/within the scaffold are illustrated in Fig. 2.

Materials loading

Based on the results obtained from some studies there are growth factors on some acellular scaffold which may enhance the healing process (Voytik-Harbin 1997; Hodde 2001). But, due to the following problems, loading of growth factors on the scaffold can make them an ideal scaffold: the finite amount of growth factors, inactivation of these bioactive factors via decellularzation process and long-term preservation, and the uncertainty of growth factors remaining uniformly at the optimal dose for regeneration process (Crapo et al. 2011; Gilbert et al. 2006; Kanematsu et al. 2003).

In addition to growth factors, other biological and non-biological materials can also be loaded on the scaffold. Biological materials such as fibronectin (Assmann 2013), hyaluronan, secreted protein acidic and rich in cysteine (SPARC) (Brown 2006c), antibody (Ye 2008) or siRNA (Vandegrift 2015) or REDV-ELP peptide (Devalliere 2018), and non-biological materials such as adhesive polymer (Brodie 2011), bio-nanocomposite (Deeken 2012) or nanostructured hydroxyapatite (Ge 2013) can impress cell behaviors, local gene modulation and scaffold properties.

The material loading is done in non-covalent and covalent approaches. Non-covalent approach or physical absorption can be direct or indirect interaction. Charge-charge interaction or existence of other secondary interaction between materials and scaffold is responsible for direct non-covalent approach; while for indirect non-covalent approach the coated intermediate biological molecule on scaffold such as heparin provides specific site to immobilization the materials. This intermediate molecule can be coated physically or chemically. But in covalent approach there is immobilization of the materials to scaffold directly through covalent bond (Lee et al. 2010).

Finally, in some cases especially in non-covalent approach of material loading, only the amount of materials which can be mixed into the scaffold should

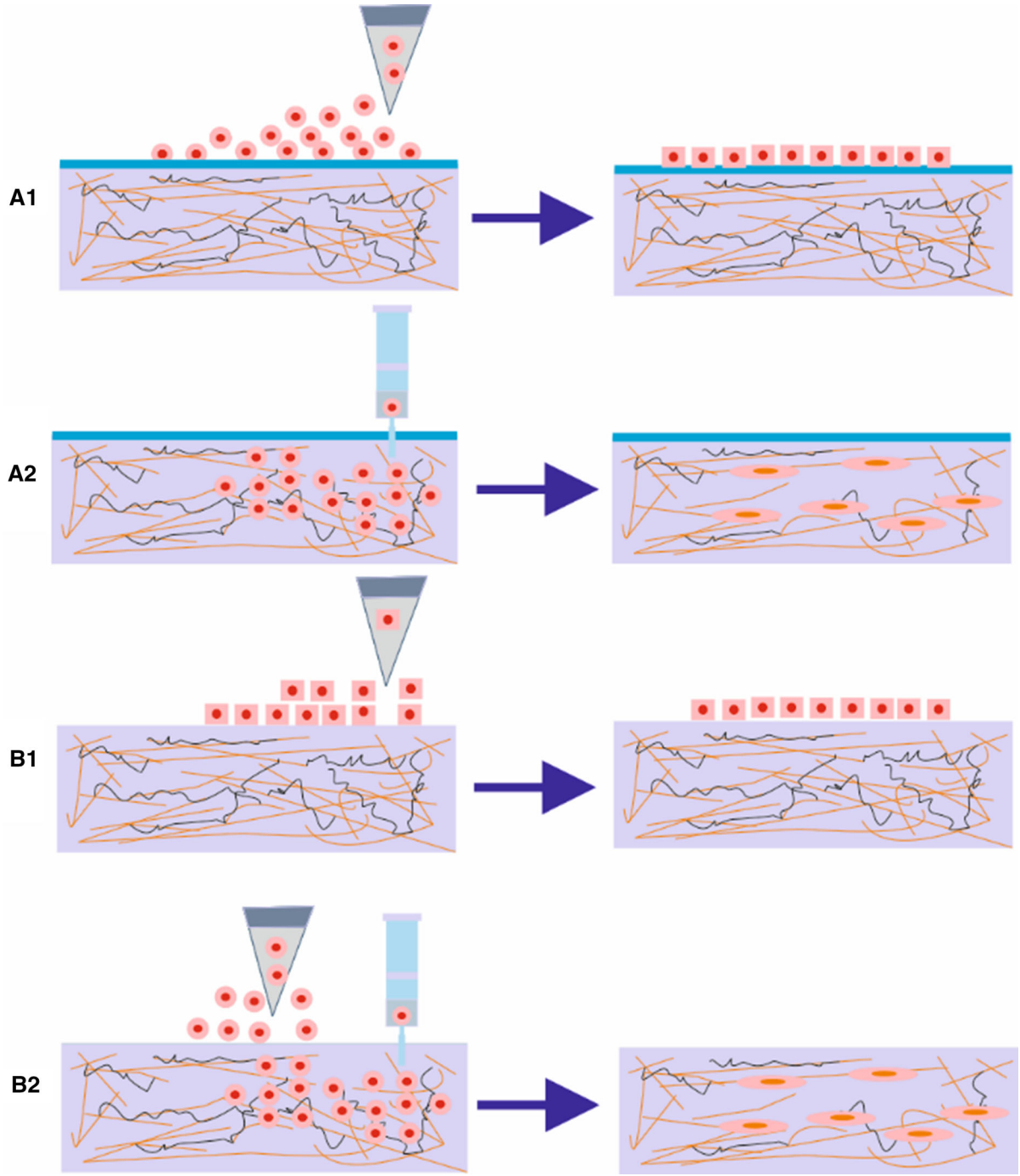

Fig. 2 Typical methods for cell seeding on/within the scaffold. **A1** epithelial/mesenchymal cell seeding on the scaffold with basement membrane, **A2** stromal/mesenchymal cell seeding within the scaffold with basement membrane, **B1** epithelial cell seeding on the scaffold without basement membrane, and **B2** stromal/mesenchymal cell seeding within the scaffold without basement membrane

not be considered, but also the material released from the scaffold is important which is named "loading capacity release kinetics" (Garg 2012). The material release from scaffold must be done in appropriate dose. So the initial part release that is termed "burst release" creates the effective therapeutic dosage (Huang and Brazel 2001), subsequently release kinetics in a time-release fashion maintains therapeutic dosage (Grassi and Grassi 2005). Typical approaches for material loading on the scaffold are illustrated in Fig. 3.

Storage

In order to achieve 'off-the-shelf' tissue-engineered scaffold and transport it from the laboratory to the clinic, we need a storage method which allows proper bio-banking of the scaffold (Schuurman 2015). In addition to all kinds of preparation methods, how the scaffold is stored is another important factor in its mechanical, structural and morphological properties and residual protein content in it (Bonenfant 2013; Wilczek 2018). Any changes to the above-mentioned features of the scaffold, in turn, may cause the following results: destruction of GAGs within tissue and subsequently decreased material reabsorption ability (Gilbert 2008; Hafeez 2005), changes in the concentration of growth factors and cytokines (Rodríguez-Ares 2009; Kim 2019; Phoomvuthisarn et al. 2019), changes in cellular attachment, rate of in vivo degradation, and infiltration, proliferation and survivability of different kind of cells (Bonenfant 2013; Freytes 2008).

Currently, several methods have been developed to preserve biological scaffolds. These techniques can be used for short-term and long-term storages with the aim of maximum preserving the component and structure of the ECM (Urbani et al. 2017). Short-term storage is used for several weeks and usually the scaffold is kept at 4 °C in storage solutions (Urbani et al. 2017; Perniconi 2011; Wagner 2014). Studies have shown that this method does not affect the mechanical and immunological properties of the scaffold for up to two months, but it is advisable to use a lower temperature to maintain more growth factors (Phoomvuthisarn et al. 2019; Jungebluth 2009). It should be noted that antibiotics are usually used in combination with storage solution such as PBS. Therefore, considering the shelf-life of antibiotics, in longer storage time, timely replacement of storage solution not be forgotten (Wagner 2014).

Lyophilization or freeze-drying, vacuum pressing and storage in liquid nitrogen are common methods used for long-term storage of biological scaffold (Badylak et al. 2009; Urbani et al. 2017). In addition to preserving the scaffold for months or years, these

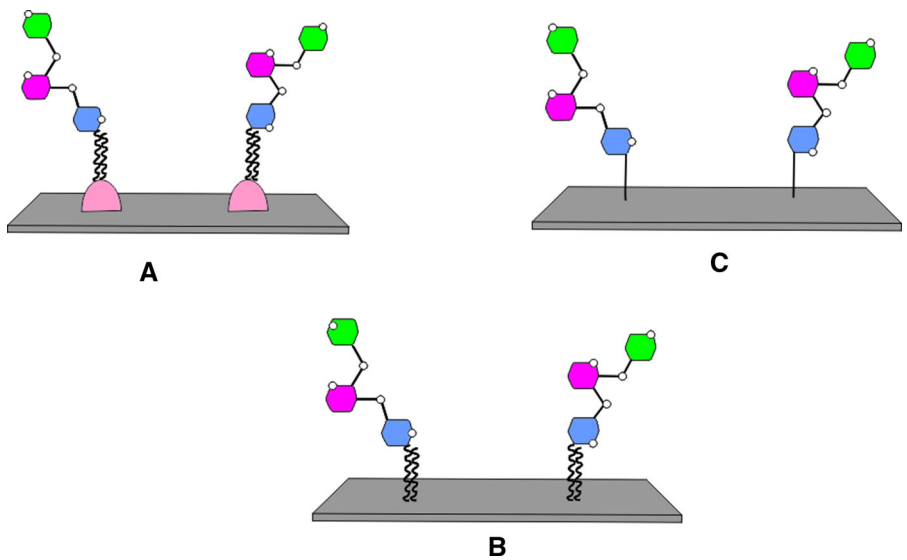

Fig. 3 Typical approaches for material loading on the scaffold. **A** Indirect non-covalent approach, **B** Direct non-covalent approach, and **B** Covalent approach

methods each have their own benefits, along with the effects they may have on the ECM. For example, in freeze-drying method, although the morphology of collage fiber and in vitro growth of cell after seeding may be altered, removing water from scaffold makes it easier to handle and transfer at room temperature (Freytes 2008; Paolin 2016); or, in vacuum pressing method, despite the change in the ultrastructural morphology of ultimate construct and the decrease in extensibility of scaffold, the several sheet of scaffold can be laminated and can be fabricated into various 3D shapes (Badylak et al. 2009; Freytes 2004,2005).

As for storage in liquid nitrogen technique, it has to be said that its storage status has a significant effect on biomechanical and morphological stability of the scaffold and slow cooled in medium (SCM) has the best effect on the preservation of the collagen/elastin/GAGs composition of the ECM (Wilczek 2018; Urbani et al. 2017). Finally, it should be noted that the effect of storage solution on the properties of scaffold should not be overlooked (Qureshi et al. 2010). Therefore, given the variety of storage methods and the advantages and disadvantages of each, the optimal method for scaffold storage should be determined based on its clinical application.

Conclusion

Selection of the best biological scaffold for safe clinical use needs multidisciplinary evaluation, which it can be demonstrated that immunologic agents, endotoxins, microorganisms and toxic residual materials from the acellularization process are eliminated, as well as structural, mechanical, functional and bioinductive properties of ECM are preserved as much as possible. After evaluating the product, it is critical to understand how storage method affects the nature of scaffold, which is important for long-term or postoperative results. Even to improve the quality of the scaffold, it is possible to seed different cell types or load biological and non-biological materials on the scaffold and give it unique features. What drives us to choose the right scaffold is our goal of using the scaffold in clinical use. With this goal in mind, it is possible to determine which of the aforementioned evaluations prevails and should focus more on, and which one is less important and even eliminable. For example, in bladder repair, the presence of scaffold's basement membrane is important so the evaluation of this part of structural property must be done. But the first step is to select a tissue as a scaffold that includes the basement membrane. Or if the sterilization method is used that is approved by the FDA, there is no need to evaluate the effectiveness of this method. Therefore, there is no requirement to perform all evaluation procedures for all types of scaffolds; and it depend on your goal, your equipment and your cost, which will lead you to careful planning for choosing the right.

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Compliance with ethical standard

Conflicts of interest The authors declare that they have no conflict of interest.

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