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Cell Responses to Surface and Architecture of Tissue Engineering Scaffolds

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1. Introduction

Tissue engineering is an interdisciplinary field that combines the knowledge and technology of cells, engineering materials, and suitable biochemical factor to create artificial organs and tissues, or to regenerate damage tissues (Langer & Vacanti, 1993). It involves cell seeding on a scaffold followed by culturing in vitro prior to implantation in vivo. The ideal scaffolds provide a framework and initial support for the cells to attach, proliferate and differentiate, and form an extracellular matrix (ECM) (Agrawal & Ray, 2001). It should be noted that scaffold surface topography and chemistry (wettability, softness and stiffness, roughness); microstructure (porosity, pore size, pore shape, interconnectivity, specific surface area) (O'Brien & Gibson, 2005b) and mechanical properties (Engler & Discher, 2006; Peyton & Putnam, 2005) have been shown to significantly influence cell behaviors such as adhesion, growth and differentiation, and to affect the bioactivity of scaffolds used for in vivo regeneration applications of various tissues, such as cartilage, skin and peripheral nerves. For tissue engineering purposes, understanding cell behavior and responses on extracellular scaffolds within physiological relevant 3D construct can aid the design of optimal bioactive tissue engineering scaffolds. Controlling cell behavior and remodeling by modulating the local engineered extracellular environment process is also a critical step in the development of the next generation of bioactive tissue engineering scaffolds. The present chapter will discuss cell responses to surface chemistry and various architecture parameters; current approaches and technologies to optimize tissue engineering scaffolds and challenges in studying the cell interaction with scaffolds.

2. Cell responses on surface chemistry of tissue engineering scaffolds

When cells adhere to surface of a scaffold, a sequence of physico -chemical reactions will happen between cells and the scaffold. Immediately after a tissue engineering scaffold is implanted into an organism or comes into contact with cell culture environments, protein adsorption to its surface occurs and which mediates the cell adhesion, and also provides signals to the cell through the cell adhesion receptors, mainly integrins. Cells can adhere on the surfaces of tissue engineering scaffolds and release active compounds for signaling, extra-cellular matrix deposition, cell proliferation and differentiation. The interaction

between cells and biomaterial scaffolds is called focal adhesion. To understand the factors that influence cell adhesive ability is a key in the development and application of new tissue engineering scaffold.

Cell attachment is a complex process, affected by numerous aspects, such as cell behavior, material surface properties, and environmental factors. Material surface properties comprise the hydrophobicity, charge, roughness, softness and chemical composition of the biomaterial surface itself.

2.1 Surface hydrophobicity

Biomaterial development has been focusing on surface modifications of biomaterials over years in order to promote a greater understanding and control of the material characteristics for regulating biocompatibility. The surface hydrophobicity is well known as a key factor to govern cell response. The surface hydrophobicity can be assessed by measuring contact angle through water spread of a droplet on a surface. The lower the contact angle, the more hydrophilic the surface is. Previous studies showed the more hydrophilic surface of material films is the much more cell adhesion on the surface (Goddard & Hotchkiss, 2007; Xu, 2007). For example, osteoblast adhesion was reported decrease when the contact angle of surface increased from 0° to 106°. Fibroblasts were found to have maximum adhesion when contact angles were between 60° and 80° (Tamada & Ikada, 1993; Wei et al., 2009). Interestingly, Vogler mentioned that the hydrophilic surface were suitable for the attachment of Madin-Darby Canine Kidney (MDCK) cells but more hydrophilic surfaces (contact angel $\theta < 65°$) did not yield progressively high level of attachment efficiency (Vogler, 1999). Furthermore, surface hydrophobicity is related to the rate of cell spreading and differentiation. On hydrophilic surfaces, cells generally showed good spreading, proliferation and differentiation. Mouse osteoblast-like cell line MC3T3-E1 showed more fractal morphology on hydrophilic surface (contact angel $\theta = 0°$) (Wei et al., 2009). 7F2 mouse osteoblasts on hydrophilic surface (contact angel $\theta = 24\text{-}31°$) demonstrated accelerated metabolic activity and osteodifferentiation compared to their unmodified counterparts (contact angel $\theta = 72°$) (Yildirim et al., 2010). The same phenomenon was observed in neuronal spreading and neurite outgrowth when the material surfaces reduced their hydrophobicity (Khorasani & Irani, 2008; Lee et al., 2003).

2.2 Protein adsorption

Since cell adhesion to material surface requires a series of cytoplasmic, transmembranal and extracellular proteins that assemble into stable contact sites (Geiger & Bendori, 1987), cell adhesion and behaviors is likely involved the adsorption onto the material surface of serum and ECM proteins (Brynda & Andrade, 1990; Hattori et al., 1985). Many proteins, including immunoglobulins, vitronectin, fibrinogen, and fibronectin (Fn), adsorb onto implant surfaces immediately upon contact with physiological fluids and modulate subsequent inflammatory responses. For example, adsorbed adhesive proteins mediate the attachment and activation of neutrophils, macrophages, and other inflammatory cells. Many literature studies mentioned that different cell behaviors, related to different hydrophobicities, may be mediated by protein absorption, because surface wettability modified the sort and the quantity of adsorbed cell adhesion molecules. Hydrophobic surfaces tend to adsorb more proteins, while hydrophilic surfaces tend to resist protein adsorption (Xu, 2007). Tamada et al. used bovine serum albumin (BSA), bovine γ-globulin and plasma Fn to study the protein

absorption onto various polymer substrates and the maximal protein absorption was observed on surfaces with water contact angle ranging from 60° to 80° (Tamada & Ikada, 1993). Hence wetting has been discredited as an adequate predictor of protein adsorption. However, there is a growing concern that surface hydrophobicity does not guarantee the protein adsorption. Certain hydrogel-like materials (e.g. oligo(ethylene glycol)) are resistant to protein adsorption even though these surfaces are (apparently) only modestly wettable (Noh & Vogler, 2006). Tamada reported that preadsorption of serum albumin prevented cell adhesion of fibroblasts to all substrates, whereas preadsorbed Fn enhanced cell adhesion of fibroblasts to all the substrates, independent of their water wettability. With preadsorption of Fn and BSA, similar pattern of cell attachment was investigated on a series of *N*-isospropylacrylamide and *N*-tert-butylacrylamide based copolymer films (Allen et al., 2006). Therefore, the composition and conformation of the adsorbed protein layer is considered to be one of the major factors in determining the nature of cell interaction with the materials.

2.3 Surface charge

After surface hydrophobicity, surface charge has been recently described a lot in the cell attachment phenomenon. Firstly, the amount of surface charges can influence cell behavior (Ishikawa et al., 2007). As the degree of charge density of poly(styrene-*ran*-acrylic acid) increased, more cell adhesion and proliferation were observed (Jung et al., 2008). Fig. 1 presented similar effect on 2-hydroxyethyl methacrylate (HEMA) and 2-methacryloxyethyl trimethyl ammonium chloride (MAETAC) copolymer hydrogels (Kim & Kihm, 2009). Secondly, many researchers reported the improved-biocompatibility, cell affinity and cell differentiation on the implanted surfaces by using the positive ions and the negative ions (Bet et al., 2003). For instance, HEMA hydrogels incorporated with positive charges supported significantly more cell attachment and spreading of osteoblasts and fibroblasts as compared to negative or neutral charges (Schneider et al., 2004). Yaszemski's groups also investigated that negatively charged oligo(poly(ethylene glycol) fumarate) hydrogels increased the extent of chondrocyte differentiation, such as collagen and glycosaminoglycan expression, in comparison with that on the neutral or positively charged hydrogel scaffolds (Dadsetan et al., 2011). Similar pattern was also performed on neuronal growth and differentiation (Makohliso et al., 1993). Positively charged coating materials such as polylysine improve neuronal attachment in vitro. On positive fluorinated ethylenepropylene (FEP) films, neurite outgrowth was significantly higher comparing to negative and uncharged substrates. Finally, the surface charges can be used to modify cell behvaior through the chemical functionalities of the polymer materials (Table 1). Lee et al. prepared polyethylene (PE) surfaces with differently chargeable functional groups (-COOH, $-CH_2OH$, $-CONH_2$ and $–CH_2NH_2$ groups) by corona discharge treatment, graft copolymerization and substitution reaction to study the effect on cell behavior (J. H. Lee et al., 1994). Results indicated that Chinese hamster ovary (CHO) cells were more adhesive to the functional group-grafted surfaces than the control PE surface due to the increased wettability by grafting hydrophilic functional groups. The best cell adhesion, growth and spreading rate were recorded on polar and positively charged surfaces (amine group-grafted PE) while the negatively charged surface (carboxylic acid group-grafted PE) still had poor growth. Moreover, the surfaces grafted with neutral amide and hydroxyl groups showed a similar number of cell attachments; however; the morphology of cells attached on

the surfaces was quite distinct. The cells were spread much more on the hydroxyl group-grafted surface than the amide group-grated one. On the other hand, surface charge may modulate protein adsorption to direct integrin binding and specificity, thereby controlling cell adhesion. Thevenot et al. mentioned that the incorporation of negative charges may facilitate adsorption of proteins which promote cell adhesion and responses (Thevenot et al., 2008). Keselowsky et al. reported that surfaces with differently chargeable functional groups (-CH_3, -OH, -COOH, and -NH_2 groups) modulated Fn adsorption and direct integrin binding and specificity to control cell adhesion of MC3T3 osteoblasts to Fn-coated surfaces followed the trend: OH >COOH =NH_2 > CH3 (Keselowsky et al., 2003). Same group also demonstrated that surfaces grafted with hydroxyl and amine groups up-regulated osteoblast-specific gene expression, alkaline phosphatase enzymatic activity, and matrix mineralization compared with surfaces presenting carboxyl and alkyl groups (Keselowsky et al., 2005). Although the molecular mechanisms in how to modulate surface charge-dependent cellular activities still remain poorly understood, these latest findings confirm that surface charge plays an important role in the application of cell biology and tissue engineering.

Functional group	Properties	Effect on cells
-CH_3	Neutral, hydrophobic	promotes increased leukocyte adhesion and phagocyte migration
-OH	Neutral, hydrophilic	increases osteoblast differentiation
-COOH	Negative, hydrophilic	Increase osteoblast attachment
-NH_2	Positive, hydrophilic	promotes myoblast and endothelial proliferation and osteoblast differentiation
–CH_2NH_2	Neutral, hydrophilic	enhance CHO attachment of Chinese hamster ovary cells

Table 1. The effect of material surface functional groups on proteins and cells (Schmidt et al., 2000)

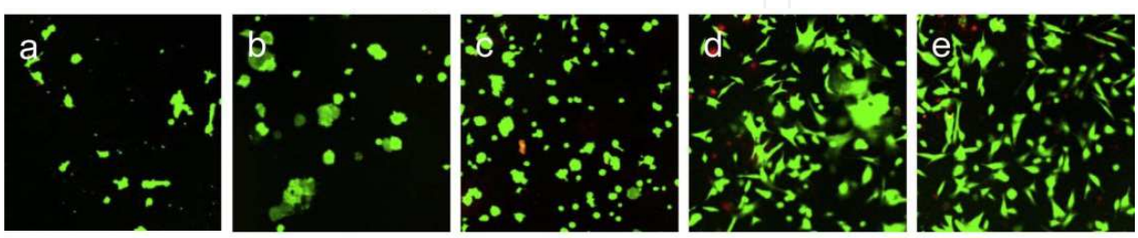

Fig. 1. Chondrocyte viability on hydrogels with different concentrations of MAETAC (positive charge functional group) in their formulation on days 1: (a) 0%; (b) 5%; (c) 10%; (d) 20%; (e) 30%. (S. Kim et al., 2009)

2.4 Surface roughness

Material surface roughness (or topography) is another important factor influencing cell adhesion and behavior. Indeed, roughness modulates the biological response of tissues in contact with the implant. Material surface roughness has a direct influence in vitro as well as in vivo on cellular morphology, proliferation, and phenotype expression. Literature papers have been reported that cells grown on microrough surfaces, were stimulated towards differentiation; as shown by their gene expression in comparison with cells growing on smooth surfaces. For instance, primary rat osteoblasts had higher proliferation and elevated alkaline phosphatase (ALP) activity and osteocalcin expression on the rough surface (0.81 μm) in comparison with smooth one (Hatano et al., 1999). In the case of human foetal osteoblastic cells (hFOB 1.19), a similar increase in cell spreading and proliferation on rough surfaces was reported (Lim, Hansen, Siedlecki, Runt, & Donahue, 2005). Depending on the scale of irregularities of the material surface, surface roughness can be divided to macroroughness (100 µm – milimeters), microroughness (100 nm – 100 µm), and nanoroughness (less than 100 nm), each with its specific influence (AGASKÁ et al., 2010). The response of cells to roughness is different depending on the cell type. For larger cells, such as osteoblasts and neurons, macroscopic descriptions of the surface roughness could be reasonable (Donoso et al, 2007). Lee et al. examined the behavior of MG63 osteoblast-like cells cultured on a polycarbonate (PC) membrane surfaces with different micropore sizes (200 nm–8.0 µm) (Lee et al., 2004). It seems that the cell adhesion and proliferation were progressively inhibited as the PC membranes had micropores with increasing size, probably due to surface discontinuities produced by track-etched pores (Fig. 2). On the other hand, increasing micropore size of the PC membrane resulted in improved cell differentiation such as higher osteocalcin expression and ALP specific activity in isolated cells. Bartolo et al. also investigated neuronal cell behavior in the surfaces with nanoscale (6.26 nm) to microscale (200 nm) roughness (Bartolo et al., 2008). The axonal length increased and the neuritis becomes highly branched on the nonoscale rough surfaces (6.26-49.38 nm). In the case of microscale rough membranes (87.2-200nm), the neurons were less developed as demonstrated by the round-shaped soma and poorly branched processes. Therefore, the nanoscale rough membranes seem to be more supportive of neurite outgrowth modulating the development process of the neurons. For smaller cells, such as human vein endothelial cells, increasing surface roughness of biomaterial surfaces at nanometer scale (10–102nm) could enhance cell adhesion and growth on roughness surfaces (Chung et al., 2003). Furthermore, Kim et al. used the dendrimer-immobilized surfaces to study nanoscale modifications and discovered that the human mammary epithelial cells (hTERT-HME1) cultured on the naked dendrimer surface (4.0 nm) were abundant in F-actin filaments of peripheral stress fibers and filopodia, compared with those cultured on the plain surface (Kim et al., 2007). However, when the surface roughness was larger than 4.0 nm, such cell stretching was inhibited, resulting in the predominant existence of round-shaped cells. Similar investigation was also reported by Dalby in the development of F-actin filaments in fibroblasts (Dalby, 2005). Interestingly, MC3T3-E1 osteoblastic cells showed that the rate of proliferation on the smooth regions (0.55nm) of the films is much greater than that on the rough regions (13nm) (Washburn et al., 2004). Therefore, the selectivity of cells on surface roughness could be highly advantage on the development of implanted devices.

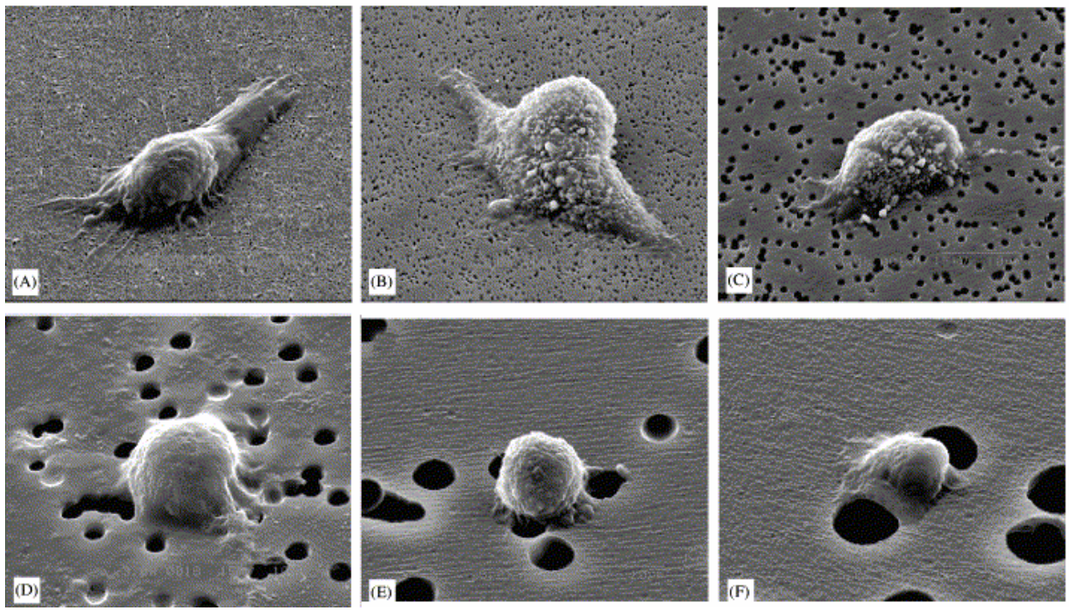

Fig. 2. SEM pictures of the MG63 cells attached on the PC membrane surfaces with different micropore sizes: (A) 0.2, (B) 0.4, (C) 1.0, (D) 3.0, (E) 5.0, and (F) 8.0 mm. (S. J. Lee et al., 2004)

2.5 Surface softness and stiffness

Surface stiffness is measure of how soft (as silk) or stiff (as rock) a materials surface is. Several studies have reported that cell attachment, proliferation and differentiation are all modulated by the substrate rigidity to a degree dependent upon the substrate stiffness in relation to the stiffness of the native tissunmee (Engler et al., 2006; Khatiwala et al., 2007). In addition, at the tissue-implant interface, cells can actively modify surfaces of the implants, altering the stiffness of microenviront of their own or other cells (Marquez et al., 2006). Tan and Teoh demonstrated that 3T3 fibroblasts preferred soft surface for proliferation. Su group used the rheometer to measure the cell adhesion force of MDCK cells on different substrate softness. The results showed that the adhesion force of MDCK cells increased with the decrease of substrate softness and which is correlated to the cell spreading area. Engler prepared polyacrylamide (PA) gels with different softness to study the correlation between cell spreading area of smooth muscle cells and elastic modulus of substrates. Cell spreading was found remarkably dependent on elastic modulus of PA gel substrates. Therefore, the softness and stiffness of substrates may regulate the mechanism between cell-ECM and seem to correlate broadly with cell adhesion response.

A limited number of attempts have yielded exciting findings (Lahann & Langer, 2005; Mrksich, 2005), in which dynamic changes of surface softness and stiffness were induced largely through application of environmental factors (e.g. temperature, pH and electric field). As a result, a study in neuronal regeneration (Jiang et al., 2008) indicted that initial softer substrates foster axonal elongation and stiffening of the substrate at a later stage could encourage outgrowth of more primary dendrites of neurons, thus promoting synaptogenesis. Moreover, cells response to mechanical alterations as demonstrated in the cell projection area and polarity was found vary depending on range of stiffness changes (Jiang et al., 2010).

3. Cell responses on architecture of tissue engineering scaffolds

On a macroscopic level, the overall shape of the scaffold provides boundaries for tissue regrowth. On a microscope level, the material provides a framework and capillary networks for local cell growth and tissue organization, permitting cell attachment, distribution and proliferation within a controllable microenvironment (Saltzman, 2002). Apart from tissue engineered skin and vascular grafts that have been progressed into clinical use, the most other tissue engineered human tissue or organs (e.g. liver and kidney) are still unsuccessful (Mikos et al., 2006). Simply produce a highly porous scaffold and cultivating it with the appropriate types of cells in most cases does not reproduce the desired feature of a normal tissue as tissue structure and function are known to be highly inter-related (Bhaia & Chen, 1999). Many tissues have a hierarchical structure that varies over length scales of 0.1-1mm (Griffith, 2002). The subcellular structures (1-10μm) control cell-cell inter-relationships and supracellular scale structures (100-1000μm) build the essential functional units of the tissue. In order to maintaining the activity of function cell, regulating cell behavior, and reconstructing 3-dimentional multicellular masses, scaffold must be optimized to satisfy cell and tissue growth including proper networks to provide fresh culture medium to all cells and remove metabolites from the cells and maintain the hierarchical cellular architectures to mimic the functional cells living environment. Altering the micro-architecture, such as the material crystallinity or the microporosity, and/or the macro-architecture of the scaffold can be achieved by changing the pores size, porosity, pore interconnectivity and tortuosity, to match the characteristics of the native tissue whilst retaining integrity (Hutmacher, 2001). A common problem encountered when using scaffolds in tissue engineering is the rapid cells attachment and proliferation on the outer edge of scaffold which restrict cell penetration to the scaffold center, resulting in a necrotic core (Freed et al., 1999). This can be addressed by altering the culture conditions use to growth tissue, for example using a flow perfusion culture system (Botchwey et al., 2001), but it is only relevant to tissue engineering in vitro. Another option or further method of addressing this is to design an optimized scaffold that will improve nutrient and cell transfer to the scaffold center, both in vitro and in vivo. As discussed in above, characterization of surface wettability, charges and softness; modification of surface chemistry by coating with adhesion molecules together with optimized the internal structure and architecture will all help to deal with this issue. Scaffold porosity in particular controls the key processes of nutrient supply to cells, metabolite dispersal, local pH stability and cell signaling. The size of the pores can affect how close the cells are at the initial stages of cultivation (allowing for cell-cell communication in three dimensions), but also influences the amount of space the cells have for 3-D organization in the later stages of tissue growth. In addition, a porous surface is known to improve mechanical interlocking between the implanted scaffolds and the surrounding natural tissue, providing greater mechanical stability at this critical interface (Karageorgiou & Kaplan, 2005). Cell seeding in the center of the scaffold and feeding the inner surfaces of the scaffolds are limited when the pores are too small whereas larger pores affect the stability of the scaffold and its ability to provide physical support for the seeded cells (Levenberg & Langer, 2004). To date cell seeding on 2-D scaffold surfaces has been shown to be easy to perform but the preparation of 3-D cell-scaffold constructs for regeneration of organs is far more complex. For example, pores of adequate size allow cells to migrate or adhere to the surface of a material, but interconnecting pores are necessary to permit cell growth into the scaffold interior.

3.1 Pore size of tissue engineering scaffold

Cell migration is modulated by a complex, spatiotemporally integrated set of biophysical mechanisms that are influenced not only by the biochemistry of extracellular and intracellular signaling, but also by the biophysics of the surrounding extracellular environment. Specific cells require different pore sizes for optimal attachment, growth and motility (Table 2) (Ranucci et al, 2000). A recent study (Yang et al., 2010)on variable pore size collagen gel found that cell migration is hindered by small pore size that invasive distance was not very sensitive in the pore size rang of 5-12μm. At small pore size, a variety of factors, including high ligand density in collagen gel that does not encourage the cell polarity and release seen in mesenchymal migration likely contributes to the limited invasion (Ulrich et al., 2010) whilst very large pore size in scaffolds have insufficient tethers on which to generate traction would also limit cell migration. As a result, many researches in tissue engineering are aimed at obtaining polymeric or bioceramic scaffolds with a very high porosity and simultaneous good control over pore size and morphology (Hou et al., 2000). The presence of pores smaller than 160μm in PLA and PLGA scaffolds, produced by salt leaching, has been reported to be optimal for attachment of human skin fibroblasts (Yang et al., 2002). Bony ingrowth was found to predominate in porous PMMA implanted in bone when the pore size was around 450μm (Ashman & Moss, 1977). Connective tissue formed when the pore size was below 100μm and extensive vascular infiltration was only observed with pores around 10000μm. In the case of polyurethane meniscal implants, structures comprising macropores (150-300 μm), highly interconnected by micropores (<50 μm) have been found to be conducive to ingrowth of fibrocartilaginous tissue (deGroot et al., 1996). The cell infiltration depth (120μm in 28 days) found in elastin scaffolds, for example, probably results from the material's high porosity and inter-connectivity (Lu, Ganesan et al., 2004). Osteoblasts was found to migrate faster inside the larger pore (100μm) of microcellular polyHIPE scaffolds; however, pore size did not affect cell penetration depth or mineralization extent (Akay et al., 2004). It has also been noticed in previous studies that cell-scaffold binding can block pores of inadequate size and geometry (Freed & Vunjak-Novakovic, 1998; Yannas, 2000). High inter-connectivity of pores is also essential to supply nutrients and allows oxygen exchange in the inner regions of a scaffold to maintain cell viability, especially for complex tissue engineering of organs.

Cell/tissue type	Optimal pore size (μm)	Scaffold material	Reference
Human skin fibroblasts	<160μm	PLA/PLG	(Yang.J et al., 2002)
Bone	450μm	PMMA	(Ashman & Moss, 1977)
Fibrocartilaginous tissue	150-300μm	Polyurethane	(deGroot et al., 1996)
Adult mammalian skin cells	20-125μm	Collagen-glycocaminoglycan	(Yannas, Lee, Orgill, SKrabut, & Murphy, 1989)
Osteogenic cells	100-150μm	Collagen-GAG	(O'Brien, Harley, Yannas, & Gibson, 2005a)
Smooth muscle cells	60-150μm	PLA	(Zeltinger, Sherwood, Graham, Mueller, & Griffith, 2001)
Endothelial cells	<80μm	Silicon nitride	(Salem et al., 2002)

Table 2. Optimal pore size for cell infiltration and host tissue ingrowth

3.2 Porosity of tissue engineering scaffold

The porosity, that is, the percentage of void volume in the materials, is also use as a means of quantifying the structure of a tissue engineering scaffolds. Researches have been focusing on the design of the scaffold to ensure appropriate porosity and porous structure for cells penetration and ingrowth. However, attempts to link scaffold porosity to cell performance have not been particularly successful. Toth et al (Toth et al., 1995) report that improvements in bone ingrowth occur with increasing porosity of macroporous biphasic calcium phosphate ceramic samples. However, they also report no discernible differences in bone union after six months implantation between scaffolds that have 30%, 50% and 70% porosity. This observation could be attributed to the complicated internal structure of scaffolds that consist of pores of different types (open, closed and blind-end pores), sizes and geometry, (Fig. 3). The presence of both random and anisotropic open porous architectures of PLA scaffolds were prepared using supercritical CO_2 aims to find the optimal channel diameter and geometry for osteosarcoma cell penetration. The results show that cells penetrate into scaffolds containing aligned channels (400μm) more extensively than those that did not.

3.3 Connectivity and tortuosity of tissue engineering scaffold

The pore structures typically consist of irregularly shaped voids and connecting channels (connects) that can be difficult to defined due to merging of adjacent cavities, resulting in the presence of fenestrations (windows) in the void walls. Beyond the fundamental requirements of adequate pore size and inter-connectivity, pore tortuosity also plays a key role in cells interaction with scaffolds. Tortuosity is defined as the ratio of the actual path length through connected pores to the Euclidean distance (shortest linear distance) (Fig.4). Tortuosity is another key factor in optimizing and designing tissue engineering scaffold which is known to influence molecules and oxygen diffusion and cell migration rate. Silva et al reported (Silva et al., 2006) that aligned channel in both hyroxyapatite (HA) and poly($_{D,L}$-lactic acid) ($P_{DL}LA$) scaffolds enhanced cell penetration and infiltration into the central region of the scaffold in comparison with tortuous channels. Analysis of human osteosarcoma cell penetration into the aligned channels revealed that cell coverage increased with increasing channel diameter from around 22% in the 170μm diameter channel to approximately 38% into the 420μm channel(Rose et al., 2004). In addition, cell penetration into 420μm channel was significantly greater than that observed within the 170μm channel. However, determination of tortuosity and cell responses on tortuous scaffold is still rarely quoted in the literature. A common method to calculate tortuosity is via the results from dissolution measurements. In this method, the tortuosity is calculated from several parameters related to the dissolution of a molecules from a matrix (Desai et al., 1966; Foster & Parrott, 1990). This approach can result in unrealistic values of more than one thousand (Papadokostaki et al., 1998) or below one (Foster & Parrott, 1990). Tortuosity can also be measured from the porosity and diffusion coefficients obtained from spin echo NMR measurements (Wu et al., 2006). Mercury intrusion porosimetry has also been suggested for determining tortuosity. Another example of tortuosity calculating is to use the inflection count metric (ICM). This approach adds the number inflections of a 3-D frame representation of a pore connecting two points and multiplies this number by the path length (Bullitt et al., 2003). Wu et al (Wu et al., 2006) described a method to find the shortest route through the pores in images of compacts using an algorithm called 'grey-weighted

distance transform (GDT)' which provide precise measurements of tortuosity. A recent study (Leber et al., 2010) showed that tortuous channels with 1 or 2 of 90˚bends had faster osteoblasts growth than the control (non-bend). It could be hypothesize that the cell sidewall affinity could have contributed to this increase in cell quantity. These observations are in harmony with studies of osteoblast alignment with parallel grooves fabricated in various material surfaces, but expand the study to tortuous channels (Ber et al., 2005). No other reports have been noticed to show the relationship between cells attachment and migration with tortuous channels.

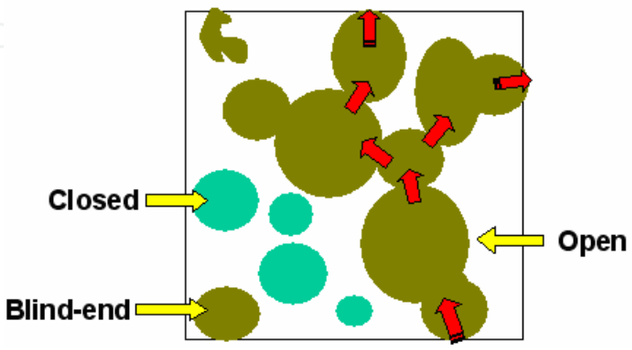

Fig. 3. Schematic of the different pore types found in tissue engineering scaffolds (Y. Wang et al., 2010)

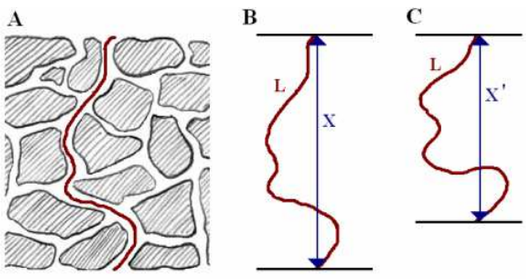

Fig. 4. Determination of tortuosity through a porous material using the arc-chord ratio (O'Connell et al., 2010)

3.4 Cell responses to dynamic scaffolds

For biodegradable polymer scaffolds, polymers slowly degrade and then dissolve following implantation. The dissolution rates represent an additional and important parameter in determining the properties of the scaffolds and can be turned to the need of specific cell and tissue. For example, cells that proliferate rapidly require scaffolds with higher degradation rates, whereas tissue structures that require stability and strength may benefit from longer-lasting material (e.g. bone, skin and tendon).

Furthermore, biodegradable polymers may provide an additional level of control over cell responses: during polymer degradation, the surface of the polymer is constantly renewed, providing a dynamic substrate for cell attachment and growth. Cells do not live in static surroundings in this situation; they exist in highly evolving dynamic environment. During cell adhesion and migration, cells adapt and communicate to their environment by

numerous methods ranging from differentiation, gene expression, growth, and apoptosis. Dynamic substrates that can alter the presentation of ligands to an attached cell will generate immediate opportunities for studies of scaffolds-cell adhesion, signaling, migration and differentiation. Dynamic substrates may also be important for generating substrates that can control the spatial and temporal interactions between two or more different populations of attached cells in tissue engineering (Yousaf, 2009). However, much less effort has been invested in studying cell responses on dynamic substrates. Currently, there is no available method to generate dynamic gradient substrates for studying cell polarity and directed cell migration. One of recent studies found that programmed erasure of substrate topography cause a decrease in cell alignment as evidenced by an increase in angular dispersion with corresponding remodeling of the actin cytoskeleton. Cell viability remained greater than 95% before and after topography change (Davis et al., 2011).

Beyond the biodegradability, mechanical input on scaffolds also significant influence the reorientation of cell shape (J. Wang et al., 2003), actin cytoskeleton remodeling (J. Wang et al., 2000) and the synthesis of extraceullar matrix (Carver et al., 1991). Many studies showed that cells are capable of surveying the external mechanical properties of their surrounding environment, respond to changes in the balance of intra- and extra- cellular forces, and regulate many important physiological proves (Pelham & Wang, 1997). As most tissue engineering scaffolds are made of biomaterials which have certain elasticity, rigidity and stretching-tension, cell responses to such scaffolds relate mechanical stimulation is becoming more and more interesting. In case of fibroblasts differentiation, mechanical stretching of silicone dishes can induce differentiation of fibroblasts into myofibrolasts, which is known to form scar tissue in vivo. Tock et al (Tock et al., 2003) reported that mechanical loading impressed α-SMA expression, a marker of myofibroblasts hat mechanical tension in granulation tissues controls myofibroblast differentiation (Hinz et al., 2001).

4. Challenges in studying cell behaviour on biomaterials and complex tissue engineering scaffolds

Cell adhesion to a material surface is an important phenomenon that controls the behavior of cells, such as their morphology, migration, growth and differentiation. Counting the cells adhered to material's surface is the most common way to evaluate a material's affinity to the cells but this method could not quantify the cell adhesion force on the material's surface. Many studies used biological procedures to measure the characteristics of adhesion between cells and biomaterials. For example, cell spreading and migration are often used as indirect indicators of adhesion strength and this lack of quantitative understanding of adhesion strength limits the interpretation of functional studies of structural and signaling adhesive components. Studying cell adhesion molecules such as focal adhesion kinase involved the binding with biomaterial's surface can provide more direct information about cell adhesion strength. However, protein expression is working on a population of cells and not a single cell due to the sensitivity of western blot or protein binding assay. Recently, a number of techniques have been developed to study the cell adhesion behavior on materials from mechanical point of view. For instance, cell adhesion strength has been studied as centrifugation force by centrifuge, tensile force by micropipette manipulation, shear force by parallel flow chamber and chemical binding force by atomic force microscope (Thoumine &

Ott, 1997; Truskey & Proulx, 1993; Leonenko et al., 2007). McClay and Lotz groups determined the cell detachment force under different speed of centrifugation and Bouafsoun et al. used flow chamber and jet impingement techniques to study cell detachment forces. However, centrifugation force by centrifuge and shear force by parallel flow chamber quantify the cell-material adhesion strength for a population of cells but not for an individual cell. Thus, tensile force by micropipette manipulation and chemical binding force by atomic force microscope (AFM) are the more suitable techniques to study the cell adhesion strength of a single cell on biomaterial surface. In 2009, Hung et al. utilized the dielectrophoresis force acting on the human bladder epithelial (ECV) cells to induce spatial movement for studying the cell adhesion strength. Dielectrophoresis is the phenomenon in which a particle, such as a living cell, is polarized and moved by the electrical gravity in a non-uniform electric field (Jones, 2005). In our study, dielectrophoresis force was also used to determine cell adhesion strength of human bladder epithelial cells on Fn and collagen type 1 coated surfaces and the cell adhesion force was similar to the one measured by AFM but much smaller than the one measured by flow chamber techniques. We suggested that the cell adhesion strength between a single cell and biomaterial surface would be different from a population of cells. Therefore, new trends and possible long-term directions for determining both adhesion process and force are highlighted.

For characterizing internal structure of tissue engineering scaffold and their correlation with cell behviour, a variety of techniques have been used to evaluate scaffold porosity including theoretical assessment, scanning electron microscopy (Flynn et al., 2006), mercury porosimetry, gas pycnometry and adsorption. SEM analysis complements the theoretical calculations of porosity (Kellomake et al., 2000; Walsh et al., 2001; Zein et al., 2002) and allows direct measurements of pore size and wall thickness and cell morphology on the surface. However, SEM cannot examine the scaffold interior without sample sectioning which introduces uncertainty due to unwanted material compression and edge effects and cells damage. Mercury porosimetry is a well known and established method, but it neither measures small mesopores (2-50nm pores) due to lack of mercury penetration nor measures very large pores as the mercury penetrates the structure before measurements can be made. The gas adsorption method is relevant to the study of porosity in nano-featured and nano-modified scaffolds (Ma, 2004), and is based on the electrical forces of attraction that bind atoms in solids. To counter the net inward attractive forces, surface atoms bind surrounding gas molecules via Van der Waals and electrical forces. Researchers have used gas adsorption to assess scaffolds with pore sizes ranging from 0.35-400 nm or 3.5 to 2000μm but the analysis does not evaluate closed pore content and cell proliferation or migration.

For analyzing the 3-D construction of tissue scaffolds and cell-material interactions, new imagining techniques such as micro-computed tomographic (micro-CT) have been developed. Feldkamp et al (Feldkamp et al., 1989) pioneered micro-CT imaging technology to analyze trabecular bone samples at a spatial resolution of 50μm. Since then, micro-CT has been used extensively in the study of bone architecture and other tissue types. Micro-CT images the specimen through exposure to small quantities of ionizing radiation and corresponding measurements of absorption. The resulting grey-scale images form a series of 2-D sequential slices which build up into a density map of the sample. With relevant computerized reconstruction, micro-CT provides precise quantitative and qualitative

information on the 3D morphology of specimens (Darling & Sun, 2004; Thurner et al., 2005; Thurner et al., 2004; Washburn et al., 2004; Williams et al., 2005) and the interior can be studied in great detail without resorting to physical sectioning or the use of toxic chemicals. Williams et al (Williams et al., 2005) recently used Micro-CT to visualize PCL scaffolds produced by selective laser sintering (SLS) and to assess the porosity and subsequent bone formation following cell seeding and implantation in mice. Micro-CT has also been used to quantify scaffold micro-architectural parameters related to compressive mechanical properties (Lin et al., 2003). Thurner et al (Thurner et al., 2005) explored X-ray Micro-CT for morphological characterization of cell cultures on filamentous 3-D scaffolds (Fig.5.) and Synchrotron Micro-CT has highlighted the subtlety of cell-scaffold interactions - fibroblasts tend to span between multi-filament yarns whereas osteoblast-like cells are confined to the filament surface (Thurner et al., 2004). Ongoing development of micro-CT techniques is improving qualitative and quantitative analysis of tissue engineering scaffolds. Jones et al (Jones et al., 2007) applied three algorithms to identify pores, interconnects and pore size distribution in bioceramic scaffolds to predict the permeability of the pore network and thus optimize bioreactor conditions for cell seeding.

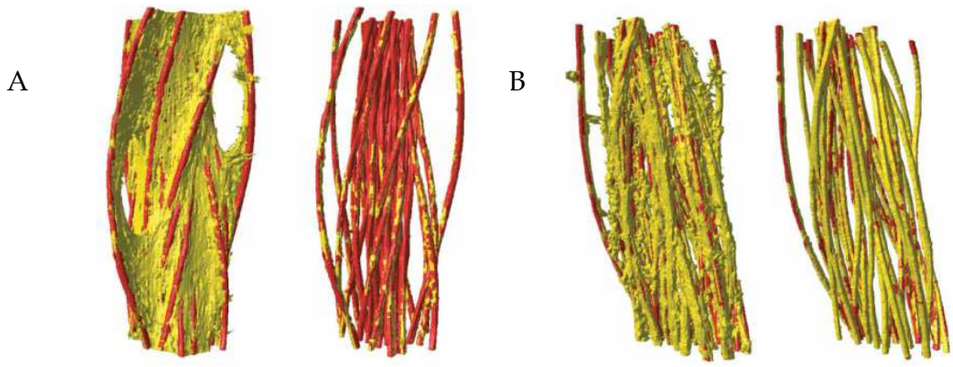

Fig. 5. 3D visualization of human foreskin fibroblasts (A) and mouse calvarial osteoblast-like cells (B). Adhesive surface (right) (both yellow) on the yarn (red) (Thurner et al., 2005)

5. Conclusion

In order to prove clinically use, tissue engineering scaffolds must consider many surface properties and 3-D structure design to maintain cell attachment, proliferation and phenotype expression. Surface and bulk mechanics, control of the scaffold interface and cell biology are essential for the development of tissue engineering. Clinical research has been demonstrating the value of tissue engineering approach on *in vivo* therapies and will likely continue to use cellular biology and signaling pathways to assess the corrections between tissue engineering and organ repair. Hence, testing cell response to tissue engineering scaffolds in vivo will provide better understanding host-implant response *in vivo* environment.

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Regenerative Medicine and Tissue Engineering - Cells and Biomaterials

Edited by Prof. Daniel Eberli

ISBN 978-953-307-663-8
Hard cover, 588 pages
Publisher InTech
Published online 29, August, 2011
Published in print edition August, 2011

Tissue Engineering may offer new treatment alternatives for organ replacement or repair deteriorated organs. Among the clinical applications of Tissue Engineering are the production of artificial skin for burn patients, tissue engineered trachea, cartilage for knee-replacement procedures, urinary bladder replacement, urethra substitutes and cellular therapies for the treatment of urinary incontinence. The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues reconstructed from readily available biopsy material induce only minimal or no immunogenicity when reimplanted in the patient. This book is aimed at anyone interested in the application of Tissue Engineering in different organ systems. It offers insights into a wide variety of strategies applying the principles of Tissue Engineering to tissue and organ regeneration.

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Hsin-I Chang and Yiwei Wang (2011). Cell Responses to Surface and Architecture of Tissue Engineering Scaffolds, Regenerative Medicine and Tissue Engineering - Cells and Biomaterials, Prof. Daniel Eberli (Ed.), ISBN: 978-953-307-663-8, InTech, Available from: http://www.intechopen.com/books/regenerative-medicine-and-tissue-engineering-cells-and-biomaterials/cell-responses-to-surface-and-architecture-of-tissue-engineering-scaffolds

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