An Overview of Mechanical Tests for Polymeric Biomaterial Scaffolds Used in Tissue Engineering

Oscar Robles-Vazquez[1], Ignacio Orozco-Avila[2], Juan C. Sánchez-Díaz[1] and Elena Hernandez[1,*]

[1]*Departamento de Ingeniería Química, CUCEI, Universidad de Guadalajara, 44430 Guadalajara, México*

[2]*Tecnología Alimentaria, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A. C., CIATEJ, 44270 Guadalajara, México*

Abstract: Mechanical characterization of polymeric biomaterial scaffolds is essential to allow biomaterials that interface with tissues and tissue engineered constructs to be developed with appropriate mechanical strength. However, the fragility of these materials makes their mechanical characterization in a quantitative manner highly challenging. Here we report an overview of testing techniques for the characterization of mechanical properties of films, membranes, hydrogels and fibers commonly used as scaffolds in tissue engineering applications.

Keywords: Hydrogel, membrane, soft material, elastic modulus, rubber elasticity, uniaxial test, compression test.

INTRODUCTION

Polymeric biomaterials used in tissue engineering fabrication are either synthetic or naturally derived. There is also a vast group of semi synthetic materials that are combinations of synthetic with natural polymers. In some cases inorganic materials are present as well. To tailor special needs, besides chemical combinations, the blending of two or more polymers allows to develop new biomaterials that exhibit combinations of properties that could not be obtained from individual polymers [1, 2].

The most common families of synthetic polymers are polyesters, polyanhydrides, and polycarbonates. Synthetic polymers offer several notable advantages over natural-origin polymers. A major advantage of synthetic polymers is that they can be tailored to suit specific functions and thus exhibit controllable properties. Furthermore, since many synthetic polymers undergo hydrolytic degradation, a scaffold's degradation rate should not vary significantly between hosts. A significant disadvantage for using synthetic polymers is that some degrade into unfavorable products, often acids. At high concentrations of these degradation products, local acidity may increase, resulting in adverse responses such as inflammation or fibrous encapsulation. Other polymeric biomaterials are natural polymers. The most common families of natural polymers are polypeptides and polysaccharides. Coming from natural sources, they usually are biocompatible and enzymatically biodegradable. The main advantage for using natural polymers is that they contain bio-functional moieties that aid the cell processes inherent to tissue engineering. However, there may be some disadvantages because depending upon the application; the previously mentioned enzymatic degradation may inhibit the very same processes that the bio-functional moieties promote. Furthermore, the rate of this degradation may not be easily controlled. Since the enzymatic activity varies between hosts, so will the degradation rate, therefore it may be difficult to determine the lifespan of natural polymers *in vivo*. Additionally, natural polymers are often weak in terms of mechanical strength (elastic modulus < 1 kPa) but cross-linking these polymers have shown to enhance their structural stability. Since many tissues undergo mechanical stresses and strains, the mechanical properties of polymeric biomaterials, synthetic or not, should be considered [1].

Tissue engineering is a multidisciplinary science that combines fundamental principles from materials engineering and molecular biology to fabricate living replacement parts for the body [3]. Scaffolds are the most common application for polymeric biomaterials in tissue engineering. The scaffold is a three-dimensional substrate that serves as a template for tissue regeneration [2]. Therefore an ideal scaffold has a three-dimensional and well defined microstructure with an interconnected pore network, possess mechanical properties similar to those of natural tissues, that is biocompatible and bio-resorbable in a convenient way [4]. The importance of mechanical properties of the biomaterials in polymeric scaffolds is that they are meant to sustain tissue meanwhile it regenerates. The

*Address correspondence to this author at the Departamento de Ingeniería Química, CUCEI, Universidad de Guadalajara, 44430 Guadalajara, México; Tel/Fax: +52 331 37 85900 ext. 27536;
E-mail: elena.hernandez@cucei.udg.mx, elena.hernandez@ymail.com

E-ISSN: 1929-5995/15

scaffold must provide, in related terms, depending upon the application, sufficient initial mechanical strength and stiffness to substitute for the mechanical function of the diseased or damaged tissue that it aims at repairing or regenerating. Scaffolds may not necessarily be required to provide complete mechanical equivalence to healthy tissue, but stiffness and strength should be sufficient to at least support and transmit forces to the host tissue site in the context [5]. In general, biomaterial polymeric scaffolds are in contact with biological fluids. It should be prevented any type of infection and immune response, blood clotting and other biological responses that could affect the properties of the fluid and, therefore, the patient. For this reason, it is important to know both host and material response for a certain biomaterial. The host response is usually related to inflammation, fibrosis, coagulation and hemolysis. The material response focuses on fracture, wear, corrosion, dissolution, swelling and leaching. As a consequence of swelling, the elastic limit of a material can be reduced leading to static fatigue or crazing [6].

Common devices used as scaffolds are membranes, hydrogels and injectable materials. Usually, membranes are considered physical barriers designed to allow permeation of specific substances. When the membranes are made of biopolymeric materials for tissue engineering applications, their three-dimensional structure is profited to enhance tissue growth and regeneration. The membranes could be networks with physical or chemical crosslinks where the porosity, hydrophilicity, water permeation, and elastic properties are tuned for applications such as wound dressings or healing patches and scaffolds for guided bone regeneration. They are useful for repair in sites where limited mechanical loading exists [6-8]. Another scaffold device is the hydrogel. Hydrogels are three-dimensional networks formed from hydrophilic homopolymers or copolymers crosslinked to form insoluble polymer matrices. These polymers, generally used above their glass transition temperature, are typically soft and elastic due to their thermodynamic compatibility with water and have found use in many biomedical applications. Hydrogels are attractive scaffolding materials because their mechanical properties can be tailored to mimic those of natural tissues. The utility of hydrogels as scaffolds is attributed to several factors, including superior biocompatibility that minimizes inflammation, thrombosis, and tissue damage, as well as high diffusivity and elasticity that parallels many tissues. As scaffolds, hydrogels are used to provide bulk and mechanical constitution to a tissue construct, whether cells are adhered to or suspended within the three-dimensional gel framework. The mechanical properties of hydrogels as tissue-engineering scaffolds can have a profound effect on attached or encapsulated cells. Tailoring of the crosslinking density is commonly used to control the properties of polymer networks, such as mechanical compliance, swelling, and mesh size. Crosslinking density can also be used to affect cells encapsulated within hydrogels [9, 10]. Besides scaffolds made of membranes or hydrogels, injectable materials are versatile scaffold devices because they eliminate the need for surgical interventions for delivery, and the minimally invasive procedure of injection. Moreover injectable scaffolds provide the ability to take the shape of the cavity in which they are placed and can thus fill irregular defects. In order to be used for a load bearing tissue for example, a scaffold must possess sufficient mechanical integrity to support the tissue, particularly during the early stages of growth [4, 11].

Here we report an overview of testing techniques for the characterization of mechanical properties of films, membranes, hydrogels and fibers commonly used as scaffolds in tissue engineering applications. Furthermore, we discuss the rheological working equations most commonly used to evaluate mechanical properties relevant to tissue engineering mechanical tests such as uniaxial extension, compression and indentation.

CHARACTERIZATION OF MECHANICAL PROPERTIES

Mechanical characterization of real biological tissue is essential to allow biomaterials that interface with tissues and tissue engineered constructs to be developed with appropriate mechanical strength. However, the fragility and viscoelasticity of these materials make their mechanical characterization in a quantitative manner highly challenging. Meanwhile the Mechanical properties of polymeric biomaterials have been under investigation to design and to control their performance in tissue engineering applications. The viscoelastic and mechanical properties of these biomaterials play an important role in their performance and durability and ultimately dictate whether the applications are successful or not. Therefore, there has been a great development of techniques for mechanically characterizing these biomaterials. Good reviews on determination of mechanical properties of

scaffolds and description of classical mechanical tests for soft polymeric biomaterials such us hydrogels are available [2, 12]. Table **1** shows an overview of published studies on polymeric scaffolds for tissue engineering where the characterization of mechanical properties is one of the concerns. The biomaterials mentioned are classified as natural and synthetic; they also represent the main component of the scaffolds when in the formulation there is more than one component. The devices are classified as films (F), membranes (M), hydrogels (H), and fibers (Fb). The standard mechanical tests presented are uniaxial tension (UT), compression (CP), indentation (IN), and dynamic mechanical (DM).

Some examples of the most common mechanical tests for biopolymeric scaffolds are discussed next. For large-scale samples, the simplest measurement that yields the elastic modulus of a specimen is the uniaxial strain test, in which the sample is grasped at two ends and pulled while axial strain and stress are simultaneously measured. In order to minimize end effects, the sample is often necked down to a lateral dimension in the central section, smaller than the ends,

Table 1: Mechanical Testing of Biopolymeric Scaffolds

Biomaterial	Device	UT	CP	IN	D
Natural					
Agarose	F				
	M			[13]	
	H	[14-17]	[14, 15]	[18]	[15]
Alginate	F			[13]	
	M		[19]	[18]	
	H	[16, 20]			[21]
Hyaluronic acid	F				
Chitosan	F	[16, 22-32]	[22, 31, 33, 34]		[30]
	M	[35-37]	[38]		[38, 39]
	H	[16, 22, 23, 40-44]	[22, 43-46]		[28, 42, 46-48]
Collagen	F	[30-32, 49-52]	[22]		[30, 53]
	M	[54-57]			[55, 58]
	H	[59-63]	[64]		[29, 53, 63, 65-68]
Gelatin	F	[22, 37, 54]	[22]		
Silk	Fb	[50]			
	F	[69, 70]			
	H	[71]	[72]		[73]
Synthetic					
Poly(l-lactic acid)	M	[25, 74]			[74]
Poly(caprolactone)	M	[75-89]	[81, 90-97]		
	H	[98-100]	[101, 102]		[103, 104]
Poly(vinyl alcohol)	H	[105-107]	[108]		
Poly(ethylene glycol)/ Polyethylene oxide	H				[29, 39, 109-113]
Polyurethane	F				[114]
	M	[115]			
Silicon	F			[116]	
	M			[117]	
Polyester	M			[118]	

Devices: film (F), membrane (M), hydrogel (H), fiber (Fb).
Mechanical tests: uniaxial tension (UT), compression (CP), indentation (IN), dynamic-mechanical (DM).

and the strain is measured directly in the necked region. Because stresses in both directions perpendicular to the axial of the specimen are zero, the elastic modulus *E* is determined by the ratio of stress to strain. Poisson's ratio *v* can be determined from the change of thickness of the specimen in the direction perpendicular to the applied stress. If the sample is anisotropic, additional uniaxial tests in the other two coordinate directions can be used. Alternatively, stresses can be applied in two (biaxial) or three (triaxial) dimensions simultaneously. These tests yield more information about the material, but do so at the cost of greater complexity [119].

The tensile properties of scaffolds made of chitosan films were tested with an Instron model 4502 at room temperature [41]. The freeze-dried samples were cut into strips of width 10 mm. Sand paper was attached to both sides of the grips to prevent the samples from breaking and the samples were mounted into the grip. The sample was thoroughly hydrated by spraying with 0.1 M PBS solution while mounted on the grip.

Sarasam *et al.* [38] studied chitosan membranes blended with poly(caprolactone) to explore changes in fatigue due to the blending process. The membranes were tested under wet 37 C conditions using an Instron testing machine under uniaxial cyclical loading. Sample preparation required good control of environmental conditions. The membranes were cut into 50 mm x 10 mm size strips, neutralized with 1 N NaOH, washed with water, and hydrated with phosphate buffer saline (PBS). A custom built chamber surrounding the grips with constant circulation of PBS maintained at 37 °C provided constant hydrating conditions [120].

Huang *et al.* [17] determined the capacity of chondrocyte- and mesenchymal stem cell (MSC)-laden agarose hydrogel constructs to achieve native tissue tensile properties (modulus, ultimate strain, and toughness) when cultured in a chemically defined medium supplemented with transforming growth factor-beta3 (TGF-b3). To evaluate tensile properties, the authors used a typical Instron Microtester to apply uniaxial tension to the samples. Since they are soft and slippery strips, they had to be seated into 120 grit sandpaper-coated grips at 25 C and moistened with PBS during the test.

For cryogels made of poly(vinylalcohol) tested by Pazos *et al.* [107], the softness and slippage of the sample specimens were a problem when using traditional clamps for uniaxial test. They came up with an original solution: the samples were attached with curved needles and 2-0 polyester braided sutures at two insertion points so gel samples slid during stretching was avoided. The strain imposed to the sample was recorded with a video-extensometer by following the displacement of four markers plotted with dye on the sample surface before the test.

These tests are most often used to measure the properties of a material in tension. However, the compressive characteristics of the scaffolds are also often of interest, especially in materials such which are to be subjected to compressive loads *in vivo*. For this purpose, specimens are generally cut into the shape of a short cylinder and compressed between two platens. In the case of swollen samples, the platens may be permeable to allow water to escape as the sample is compressed, thereby obtaining information on the permeability of the sample from the time-dependent compression following the application of a load. Both confined and unconfined compression tests are useful. The advantage of confined compression, in which the sample is placed in a rigid cylindrical chamber, typically with no permeable side walls, is that the stress strain, and flow of water are purely axial and the results can be more easily interpreted [119].

Scott *et al.* [116] developed an indentation method of characterizing freestanding silicon films with finite-sized indenters that turned out to be versatile, since it can be applied to tests with large ranges in stiffness. Using finite-sized indenters they achieved greater control over imposed strain, they found that to be highly advantageous when testing very thin films. The test was particularly useful to characterizing soft materials that present gripping and strain-instrumentation challenges, such as elastomers and biological materials. The test arrangement is a circular film with fixed outer edge that is indented by finite-sized spheres. The applied displacement and resulting load are the measured quantities that allow determination of mechanical properties from load–deflection relationships.

As an example of measurement of the adhesive indentation behaviors is the work by Ju *et al.* [117] where they presented the characterization of a square of silicone rubber membrane deformed by a finely polished flat-ended cylinder (or indenter) under a small deformation (ca. 10% in strain). An apparatus was constructed to allow simultaneously measurements of the indenter displacement and the applied force. A

number of interesting phenomena such as the "jump-into-contact" and the "pull-off" between the membrane and the moving cylinder were observed and the loading-unloading force displacement curves (compliance) were measured. Upon loading when the indenter-membrane gap decreased, the membrane jumped into contact with the indenter surface. During unloading, a negative tensile force was needed to separate the indenter from the membrane. The resultant measured force is a sum of contributions from the interfacial forces and the mechanical reaction of the elastic medium. The former has an origin in van der Waals and electrostatic interactions, while the latter is mainly governed by bulk mechanical properties such as the Young's modulus and Poisson's ratio of the membrane materials. With this kind of test, the "jump-into-contact" and "pull-off" events that are well known in colloid and surface science can be evaluated for soft membranes common in tissue engineering.

Ahearne *et al.* [18] present an indentation method for characterizing the viscoelastic properties of alginate and agarose hydrogel based constructs, which are often used as a model system of soft biological tissues. A sensitive long working distance microscope was used for measuring the time-dependent deformation of the thin circular hydrogel membranes under a constant load. The deformation of the constructs was measured laterally. This is a convenient technique for soft tissue engineering that allows measuring mechanical properties of hydrogels in a non-destructive, online and real time fashion.

MECHANICAL ANALYSIS

The mechanical behavior of polymeric biomaterial scaffolds for tissue engineering applications is best understood using the theories of rubber elasticity. These theories are based on time-independent and time-dependent recovery of the chain orientation and structure, respectively. By using theories to describe the mechanical behavior, it is possible to analyze the polymeric structure. It is also possible, and sometimes necessary, to use theories to extrapolate mechanical properties to conditions in which the material may be used. In many instances it is not possible to test the scaffolds under the exact conditions in which the device is used. For these applications it is of particular importance to use theories to extrapolate properties to these conditions [12].

Rubbers are materials that respond to stresses with nearly instantaneous and fully reversible deformation. General characteristics of rubber elastic behavior include high extensibility generated by low mechanical stress, complete recovery after removal of the deformation, and high extensibility and recovery that are driven by entropic rather than enthalpic changes [121].

The elasticity of a material is typically characterized by its stress–strain relationship, where the stress is the force acting per unit area and the strain is the fractional change in length of the specimen. In the case of uniaxial stress, a material might exhibit a stress–strain relation linear (or nearly so) for small strains, but becomes non-linear as strains increase above a certain level. The elastic, or Young's, modulus *E* is defined as the ratio of stress to strain; it would be constant for small strains, but eventually would increase as the material experiences increasing strain. The elastic modulus of most biologic tissues is highly non-linear [119], hence the scaffolds designed for tissue engineering applications are to exhibit an increasing elastic modulus for higher strains (Figure **1**).

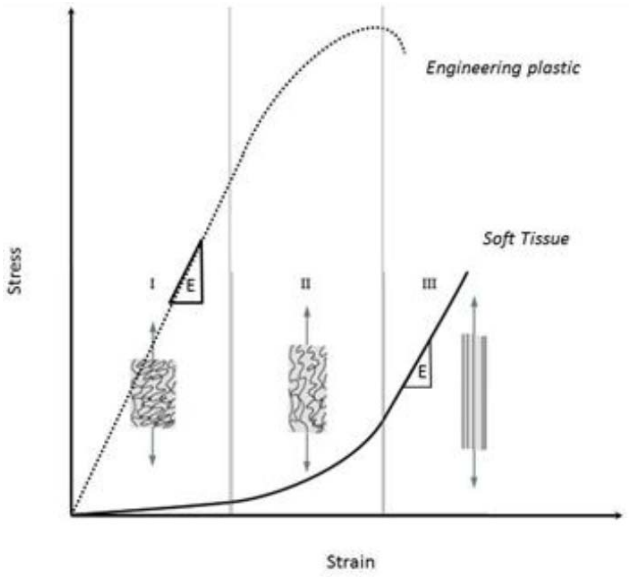

Figure 1: Schematic diagram of a typical stress-strain curve for an engineering plastic and a soft tissue showing fiber morphology changes responsible for the increment in the elastic modulus E.

Being the scaffolds complex materials, it may happen, besides elastic deformation, that plastic deformation comes into play; the material experiences an irreversible deformation, usually at high levels of stress, and fails to return to its original length when the stress is removed.

In the analysis of the indentation test for freestanding films presented by Scott *et al.* [116] where

the elasticity of the film is the primary concern, the relevant variables are load magnitude *P*, total deflection of indenter δ, freestanding span radius *a*, indenter radius *R*, and film thickness *h*; the relevant material properties are elastic modulus *E* and the Poisson's ratio *v*. The relationship *h*/*a* allows determining the mechanical response of the film that is if the film will behave as a plate, as a membrane or will exhibit a behavior somewhere in between. The analysis for the plate regime was limited to small deformations to determine the elastic modulus. When modeling the total deflection of the indenter for the case of relatively soft materials the authors considered necessary to account for two contributions: the overall plate deflection of the clamped circular film and the indenter penetration to the plate since both deflections are comparable in magnitude. The total deflection of the indenter was then estimated by treating the system as two springs in series, in which case the penetration of the indenter and deflection of the plate superpose:

$$\delta = \left(\frac{9P^2}{16RE_*^{\ 2}}\right)^{1/3} + \left(\frac{3Pa^2\left(1-v^2\right)}{4\pi Eh^3}\right) \tag{1}$$

where E_* is defined as a weighted average of properties of the indenter (1) and the plate (2)

$$\frac{1}{E_*} = \frac{1-v_1}{E_1} + \frac{1-v_2}{E_2} \tag{2}$$

On the other hand, in the analysis for the membrane regime, they present two theoretical models for the membrane regime; one corresponds to a modified version of the classical Schwerin solution for point loads, while the other explicitly deals with contact. For the load–deflection relationship, the modified version of the classical Schwerin point-load solution for membranes with *v* = 1/3 is:

$$\delta = f\left(v\right)a\left(\frac{P}{Eah}\right)^{1/3} \tag{3}$$

where

$$f(v) \approx 1.049 - 0.146v - 0.158v^2 \tag{4}$$

For a more accurate determination of the load–deflection relationship, the authors use the following equation for a membrane with a Poisson = 1/2, zero pre-strain, small rotations, and negligible radial displacements compared with downward displacements:

$$\frac{\delta}{R} = \left(\frac{16}{9\pi}\right)^{1/3}\left(\frac{a}{R}\right)^{3/4}\left(\frac{P}{EhR}\right)^{1/3} \tag{5}$$

Both equations have the same power-law relationship between load and displacement, but a different dependence on span and indenter radius.

For extremely soft samples, an adhesive indentation test is preferred. A theoretical analysis using linear elasticity was presented by Ju *et al.* [117] to fit the load-displacement curve (compliance) originated in an adhesive indentation test and thus estimated the Young's modulus of a micro-fabricated silicone rubber membrane. In the analysis it is considered that when an external force *F* is applied to a clamped square film *via* a cylindrical punch, the membrane deforms to a central deflection w_0. Since the diameter of the cylindrical punch is small compared to the film dimension *a*, the indentation force is appropriately approximated to a central point force. A simple elastic model based on pure bending of a square plate clamped at the edges gives as a result that at equilibrium, the linear constitutive relation is given by

$$w_0 = \vartheta\frac{Fa^2}{\kappa} \tag{6}$$

where ϑ is the numerical factor depending on the membrane geometry, loading type (e.g. central load), and support configuration (e.g. clamped edges). The parameter κ is the bending rigidity defined by the authors to be

$$\kappa = \frac{Eh^3}{12\left(1-v^2\right)} \tag{7}$$

with *E* and the elastic modulus and the Poisson's ratio, respectively. Their analysis is valid only for small-strain deformation whereby stretching stress is negligible.

To determine the elastic modulus of fully swollen hydrogels, Ahearne *et al.* [18] present one application of the large deformation theory based on Mooney–Rivlin elasticity. The authors have already determined that fully swollen hydrogels exhibit rubber like characteristics and that their mechanical behaviors can be characterized using Mooney–Rivlin equations. The application of the model basically consist of describing the deformation of a membrane by monitoring the central displacement δ due to the weight *w* of a ball of radius *R* to find the Young's modulus *E* of the membrane of thickness *h* and radius *a*.

$$\frac{6w}{EhR} = 0.075\left(\frac{\delta}{R}\right)^2 + 0.78\left(\frac{\delta}{R}\right) \quad (8)$$

This equation has been developed for a ball and a sample with the dimensional characteristics of a/R = 5 and $\delta/R \leq 1.7$. This model also assumes that for the membrane the ratio h/a is small and the deformation is large, hence stretching dominates over bending.

Illustrating another example for the application of the Mooney-Rivlin equations, Susilo *et al.* [57] worked on a three dimensional model of a collagen fibril matrix this time undergoing uniaxial tensile stress. They run simulations based on a unit cell model where fibrils were arranged to simulate an extra cellular matrix testing both the general case of Mooney-Rivlin equations presented by Ahearne *et al.* [18] and the particular case of constitutive equations for neo-Hookean materials where the higher order terms could be neglected.

Since hydrogel scaffolds for tissue engineering are typically water-swollen to maintain proliferating cells, it is necessary to account for the rubber elasticity and swelling phenomena simultaneously [9]. Peppas and Merrill [122] modified the original theories for polymer elasticity developed by Flory [123] to account for hydrogels tested in the presence of a solvent. The applied stress τ as a function of elongation α is given by

$$\tau = \frac{\rho RT}{\bar{M}_c}\left(1 - \frac{2\bar{M}_c}{\bar{M}_N}\right)\left(\alpha - \frac{1}{\alpha^2}\right)\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} \quad (9)$$

where ρ is the polymer density, R is the universal gas constant, T is absolute temperature; relevant parameters to polymer structure are the average molecular weight between crosslinks $\bar{M}_c$ and the number average molecular weight $\bar{M}_N$; experimental values are the swollen polymer volume fractions $v_{2,i}$. This equation works well for small deformation test. If predictions at higher elongations are required, the Mooney-Rivlin equation modified to better describe the behavior of swollen hydrogels presented by Anseth *et al.* [12] is recommended

$$\tau_s = 2C_1 v_r^{1/3}\left(\alpha - \frac{1}{\alpha^2}\right) + 2C_2 v_r^{5/3}\left(1 - \frac{1}{\alpha^3}\right) \quad (10)$$

where C_1 and C_2 are fitting constants.

CLOSING REMARKS

Generally, a biomaterial polymeric scaffold for tissue engineering applications should have sufficient mechanical strength to maintain integrity until the new tissue regenerates, maintain the space for cell ingrowths and nutrient transport *in vitro* and support physiological loadings *in vivo*. The scaffold should match its mechanical properties to that of the native tissue to both prevent stress shielding and give the cells proper mechanical cues as the ones they normally receive in their native environment. Although there are plenty of works on mechanical characterization of biopolymeric materials and tissue engineering devices, really few reports are on modeling the performance of such devices for a bottom-up design. However, what there are missing appropriate enough models that relate the scaffold structure and material properties, to their mechanical behavior. The establishment of these relationships will provide the foundation to develop better polymeric systems to help design suitable customized scaffolds.

REFERENCES

[1] Yoon DM, Fisher JP. Polymeric scaffolds for tissue engineering applications. In: Fisher JP, Mikos AG, Bronzino JD, editors. Tissue Engineering. Boca Raton: CRC Press; 2007; p. 8.1-8.18. http://dx.doi.org/10.1201/9781420008333.sec2

[2] Liu C, Xia Z, Czernuszka JT. Design and development of three-dimensional scaffolds for tissue engineering. Chem Eng Res Des 2007; 85: 1051-64. http://dx.doi.org/10.1205/cherd06196

[3] Khademhosseini A, Vacanti JP, Langer R. Progress in tissue engineering. Sci Am 2009; 300: 64-100. http://dx.doi.org/10.1038/scientificamerican0509-64

[4] Biondi M, Ungaro F, Quaglia F, Netti PA. Controlled drug delivery in tissue engineering. Adv Drug Deliv Rev 2008; 60: 229-42. http://dx.doi.org/10.1016/j.addr.2007.08.038

[5] Hutmacher D, Woodfield T, Dalton P, Lewis J. Scaffold design and fabrication. In: van Blitterswijk C, Thomsen P, Lindahl A, *et al.*, editors. Tissue Engineering. Academic Press Series in Biomedical Engineering. London: Academic Press; 2008. p. 403-54. http://dx.doi.org/10.1016/b978-0-12-370869-4.00014-8

[6] Stamatialis DF, Papenburg BJ, Girons M, *et al.* Medical applications of membranes: Drug delivery, artificial organs and tissue engineering. J Memb Sci 2008; 308: 1-34. http://dx.doi.org/10.1016/j.memsci.2007.09.059

[7] Temtem M, Silva LMC, Andrade PZ, *et al.* Supercritical CO2 generating chitosan devices with controlled morphology. Potential application for drug delivery and mesenchymal stem cell culture. J Supercrit Fluids 2009; 48: 269-77. http://dx.doi.org/10.1016/j.supflu.2008.10.020

[8] Dimitru S. Polymeric Biomaterials. 2nd ed: Marcel Decker; 2002.

[9] Slaughter BV, Khurshid SS, Fisher OZ, Khademhosseini A, Peppas NA. Hydrogels in regenerative medicine. Adv Mater 2009; 21: 3307-29. http://dx.doi.org/10.1002/adma.200802106

[10] Hou Y, Schoener CA, Regan KR, Munoz-Pinto D, Hahn MS, Grunlan MA. Photo-Cross-linked PDMS star-PEG hydrogels: synthesis, characterization, and potential application for tissue engineering scaffolds. Biomacromolecules 2010; 11: 648-56. http://dx.doi.org/10.1021/bm9012293

[11] Kretlow JD, Klouda L, Mikos AG. Injectable matrices and scaffolds for drug delivery in tissue engineering. Adv Drug Deliv Rev 2007; 59: 263-73. http://dx.doi.org/10.1016/j.addr.2007.03.013

[12] Anseth KS, Bowman CN, Brannon-Peppas L. Mechanical properties of hydrogels and their experimental determination. Biomaterials 1996; 17: 1647-57. http://dx.doi.org/10.1016/0142-9612(96)87644-7

[13] Ahearne M, Siamantouras E, Yang Y, Liu KK. Mechanical characterization of biomimetic membranes by micro-shaft poking. J R Soc Interface 2009; 6: 471-8. http://dx.doi.org/10.1098/rsif.2008.0317

[14] Buckley CT, Thorpe SD, O'Brien FJ, Robinson AJ, Kelly DJ. The effect of concentration, thermal history and cell seeding density on the initial mechanical properties of agarose hydrogels. J Mech Behav Biomed Mater 2009; 2: 512-21. http://dx.doi.org/10.1016/j.jmbbm.2008.12.007

[15] Hafemann B, Ensslen S, Erdmann C, *et al*. Use of a collagen/elastin-membrane for the tissue engineering of dermis. Burns 1999; 25: 373-84. http://dx.doi.org/10.1016/S0305-4179(98)00162-4

[16] Majima T, Funakosi T, Iwasaki N, *et al*. Alginate and chitosan polyion complex hybrid fibers for scaffolds in ligament and tendon tissue engineering. J Orthop Sci 2005; 10: 302-7. http://dx.doi.org/10.1007/s00776-005-0891-y

[17] Huang AH, Yeger-McKeever M, Stein A, Mauck RL. Tensile properties of engineered cartilage formed from chondrocyte- and MSC-laden hydrogels. Osteoarthr Cartil 2008; 16: 1074-82. http://dx.doi.org/10.1016/j.joca.2008.02.005

[18] Ahearne M, Yang Y, El Haj AJ, Then KY, Liu KK. Characterizing the viscoelastic properties of thin hydrogel-based constructs for tissue engineering applications. J R Soc Interface 2005; 2: 455-63. http://dx.doi.org/10.1098/rsif.2005.0065

[19] Nguyen VB, Wang CX, Thomas CR, Zhang Z. Mechanical properties of single alginate microspheres determined by microcompression and finite element modelling. Chem Eng Sci 2009; 64: 821-9. http://dx.doi.org/10.1016/j.ces.2008.10.050

[20] Chou AI, Akintoye SO, Nicoll SB. Photo-crosslinked alginate hydrogels support enhanced matrix accumulation by nucleus pulposus cells *in vivo*. Osteoarthr Cartil 2009; 17: 1377-84. http://dx.doi.org/10.1016/j.joca.2009.04.012

[21] Banerjee A, Arha M, Choudhary S, *et al*. The influence of hydrogel modulus on the proliferation and differentiation of encapsulated neural stem cells. Biomaterials 2009; 30: 4695-9. http://dx.doi.org/10.1016/j.biomaterials.2009.05.050

[22] Hsieh WC, Chang CP, Lin SM. Morphology and characterization of 3D micro-porous structured chitosan scaffolds for tissue engineering. Colloids Surf B Biointerfaces 2007; 57: 250-5. http://dx.doi.org/10.1016/j.colsurfb.2007.02.004

[23] Depan D, Kumar AP, Singh RP. Cell proliferation and controlled drug release studies of nanohybrids based on chitosan-g-lactic acid and montmorillonite. Acta Biomater 2009; 5: 93-100. http://dx.doi.org/10.1016/j.actbio.2008.08.007

[24] Wan Y, Wu Q, Wang S, Zhang SM, Hu ZL. Mechanical properties of porous polylactide/chitosan blend membranes. Macromol Mater Eng 2007; 292: 598-607. http://dx.doi.org/10.1002/mame.200600481

[25] Duan B, Yuan XY, Zhu Y, *et al*. A nanofibrous composite membrane of PLGA-chitosan/PVA prepared by electrospinning. Eur Polym J 2006; 42: 2013-22. http://dx.doi.org/10.1016/j.eurpolymj.2006.04.021

[26] Thierry B, Merhi Y, Silver J, Tabrizian M. Biodegradable membrane-covered stent from chitosan-based polymers. J Biomed Mater Res A 2005; 75: 556-66. http://dx.doi.org/10.1002/jbm.a.30450

[27] Liu Y, Vrana NE, Cahill PA, McGuinness GB. Physically Crosslinked Composite Hydrogels of PVA With Natural Macromolecules: Structure, Mechanical Properties, and Endothelial Cell Compatibility. J Biomed Mater Res B 2009; 90: 492-502. http://dx.doi.org/10.1002/jbm.b.31310

[28] Costa ED, Mansur HS. Preparation and characterization of chitosan/poly(vinyl alcohol)blend chemically crosslinked by glutaraldehyde for tissue engineering application. Quimica Nova 2008; 31: 1460-6.

[29] Ma D, Zhang LM, Yang C, Yan L. UV photopolymerized hydrogels with beta-cyclodextrin moieties. J Polym Res 2008; 15: 301-7. http://dx.doi.org/10.1007/s10965-007-9171-1

[30] Rafat M, Li FF, Fagerholm P, *et al*. PEG-stabilized carbodiimide crosslinked collagen-chitosan hydrogels for corneal tissue engineering. Biomaterials 2008; 29: 3960-72. http://dx.doi.org/10.1016/j.biomaterials.2008.06.017

[31] Wan Y, Fang Y, Wu H, Cao XY. Porous polylactide/chitosan scaffolds for tissue engineering. J Biomed Mater Res A 2007; 80: 776-89. http://dx.doi.org/10.1002/jbm.a.31025

[32] Wang XH, Yan YN, Xiong Z, *et al*. Preparation and evaluation of ammonia-treated collagen/chitosan matrices for liver tissue engineering. J Biomed Mater Res B 2005; 75: 91-8. http://dx.doi.org/10.1002/jbm.b.30264

[33] Duan B, Wu LL, Yuan XY, *et al*. Hybrid nanofibrous membranes of PLGA/chitosan fabricated *via* an electrospinning array. J Biomed Mater Res A 2007; 83: 868-78. http://dx.doi.org/10.1002/jbm.a.31408

[34] Correlo VM, Boesel LF, Pinho E, *et al*. Melt-based compression-molded scaffolds from chitosan-polyester blends and composites: morphology and mechanical properties. J Biomed Mater Res A 2009; 91: 489-504. http://dx.doi.org/10.1002/jbm.a.32221

[35] Madihally SV, Matthew HWT. Porous chitosan scaffolds for tissue engineering. Biomaterials 1999; 20: 1133-42. http://dx.doi.org/10.1016/S0142-9612(99)00011-3

[36] Tang YF, Du YM, Li Y, Wang XY, Hu XW. A thermosensitive chitosan/poly(vinyl alcohol) hydrogel containing hydroxyapatite for protein delivery. J Biomed Mater Res A 2009; 91: 953-63. http://dx.doi.org/10.1002/jbm.a.32240

[37] Nagahama H, Maeda H, Kashiki T, Jayakumar R, Furuike T, Tamura H. Preparation and characterization of novel chitosan/gelatin membranes using chitosan hydrogel. Carbohydr Polym 2009; 76: 255-60. http://dx.doi.org/10.1016/j.carbpol.2008.10.015

[38] Sarasam A, Madihally SV. Characterization of chitosan-polycaprolactone blends for tissue engineering applications. Biomaterials 2005; 26: 5500-8. http://dx.doi.org/10.1016/j.biomaterials.2005.01.071

[39] Caridade SG, da Silva RMP, Reis RL, Mano JF. Effect of solvent-dependent viscoelastic properties of chitosan membranes on the permeation of 2-phenylethanol. Carbohydr Polym 2009; 75: 651-9. http://dx.doi.org/10.1016/j.carbpol.2008.09.011

[40] Suh J-KF, Matthew HWT. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. Biomaterials 2000; 21: 2589-98. http://dx.doi.org/10.1016/S0142-9612(00)00126-5

[41] Adekogbe I, Ghanem A. Fabrication and characterization of DTBP-crosslinked chitosan scaffolds for skin tissue engineering. Biomaterials 2005; 26: 7241-50. http://dx.doi.org/10.1016/j.biomaterials.2005.05.043

[42] Hoffmann B, Seitz D, Mencke A, Kokott A, Ziegler G. Glutaraldehyde and oxidised dextran as crosslinker reagents

for chitosan-based scaffolds for cartilage tissue engineering. J Mater Sci Mater Med 2009; 20: 1495-503. http://dx.doi.org/10.1007/s10856-009-3707-3

[43] Kong L, Ao Q, Wang A, *et al*. Preparation and characterization of a multilayer biomimetic scaffold for bone tissue engineering. J Biomater App 2007; 22: 223-9. http://dx.doi.org/10.1177/0885328206073706

[44] van Blitterswijk C, Thomsen P, Lindahl A, *et al*., editors. Controlled release strategies in tissue engineering. London: Academmic Press; 2008.

[45] Abdel-Fattah WI, Jiang T, El-Bassyouni GET, Laurencin CT. Synthesis, characterization of chitosans and fabrication of sintered chitosan microsphere matrices for bone tissue engineering. Acta Biomater 2007; 3: 503-14. http://dx.doi.org/10.1016/j.actbio.2006.12.004

[46] Funakoshi T, Majima T, Iwasaki N, *et al*. Application of tissue engineering techniques for rotator cuff regeneration using a chitosan-based hyaluronan hybrid fiber scaffold. Am J Sports Med 2005; 33: 1193-201. http://dx.doi.org/10.1177/0363546504272689

[47] Hong Y, Gong YH, Gao CY, Shen JC. Collagen-coated polylactide microcarriers/chitosan hydrogel composite: injectable scaffold for cartilage regeneration. J Biomed Mater Res A 2008; 85: 628-37. http://dx.doi.org/10.1002/jbm.a.31603

[48] Park KM, Joung YK, Park KD, Lee SY, Lee MC. RGD-conjugated chitosan-Pluronic hydrogels as a cell supported scaffold for articular cartilage regeneration. Macromol Res 2008; 16: 517-23. http://dx.doi.org/10.1007/BF03218553

[49] Sahoo S, Cho-Hong JG, Siew-Lok T. Development of hybrid polymer scaffolds for potential applications in ligament and tendon tissue engineering. Biomed Mater 2007; 2: 169-73. http://dx.doi.org/10.1088/1748-6041/2/3/001

[50] Lawrence BD, Marchant JK, Pindrus MA, Omenetto FG, Kaplan DL. Silk film biomaterials for cornea tissue engineering. Biomaterials 2009; 30: 1299-308. http://dx.doi.org/10.1016/j.biomaterials.2008.11.018

[51] Vrana NE, Elsheikh A, Builles N, Damour O, Hasirci V. Effect of human corneal keratocytes and retinal pigment epithelial cells on the mechanical properties of micropatterned collagen films. Biomaterials 2007; 28: 4303-10. http://dx.doi.org/10.1016/j.biomaterials.2007.06.013

[52] Yokoya S, Mochizuki Y, Nagata Y, Deie M, Ochi M. Tendon-bone insertion repair and regeneration using polyglycolic acid sheet in the rabbit rotator cuff injury model. Am J Sports Med 2008; 36: 1298-309. http://dx.doi.org/10.1177/0363546508314416

[53] McHale MK, Setton LA, Chilkoti A. Synthesis and *in vitro* evaluation of enzymatically cross-linked elastin-like polypeptide gels for cartilaginous tissue repair. In: van Blitterswijk C, Thomsen P, Lindahl A, *et al*., editors. Tissue Engineering. 112005. p. 1768-79.

[54] Beachley V, Wen XJ. Fabrication of nanofiber reinforced protein structures for tissue engineering. Mater Sci Eng C 2009; 29: 2448-53. http://dx.doi.org/10.1016/j.msec.2009.07.008

[55] Chan BP, So KF. Photochemical crosslinking improves the physicochemical properties of collagen scaffolds. J Biomed Mater Res A 2005; 75: 689-701. http://dx.doi.org/10.1002/jbm.a.30469

[56] Song JH, Kim HE, Kim HW. Collagen-apatite nanocomposite membranes for guided bone regeneration. J Biomed Mater Res B 2007; 83: 248-57. http://dx.doi.org/10.1002/jbm.b.30790

[57] Susilo ME, Roeder BA, Voytik-Harbin SL, Kokini K, Nauman EA. Development of a three-dimensional unit cell to model the micromechanical response of a collagen-based extracellular matrix. Acta Biomater 2010; 6: 1471-86. http://dx.doi.org/10.1016/j.actbio.2009.11.014

[58] Xu CC, Chan RW, Tirunagari N. A biodegradable, acellular xenogeneic scaffold for regeneration of the vocal fold lamina propria. In: van Blitterswijk C, Thomsen P, Lindahl A, *et al*., editors. Tissue Engineering. 132007. p. 551-66.

[59] Slovikova A, Vojtova L, Jancar J. Preparation and modification of collagen-based porous scaffold for tissue engineering. Chem Papers 2008; 62: 417-22. http://dx.doi.org/10.2478/s11696-008-0045-8

[60] Liu WG, Deng C, McLaughlin CR, *et al*. Collagen-phosphorylcholine interpenetrating network hydrogels as corneal substitutes. Biomaterials 2009; 30: 1551-9. http://dx.doi.org/10.1016/j.biomaterials.2008.11.022

[61] Ahearne M, Yang Y, Then KY, Liu KK. Non-destructive mechanical characterisation of UVA/riboflavin crosslinked collagen hydrogels. Br J Ophthalmol 2008; 92: 268-71. http://dx.doi.org/10.1136/bjo.2007.130104

[62] Brigham MD, Bick A, Lo E, Bendali A, Burdick JA, Khademhosseini A. Mechanically robust and bioadhesive collagen and photocrosslinkable hyaluronic acid semi-interpenetrating networks. Tissue Eng Part A 2009; 15: 1645-53. http://dx.doi.org/10.1089/ten.tea.2008.0441

[63] Tan RW, Niu XF, Gan SL, Feng QL. Preparation and characterization of an injectable composite. J Mater Sci Mater Med 2009; 20: 1245-53. http://dx.doi.org/10.1007/s10856-009-3692-6

[64] Ng KW, Saliman JD, Lin EY, *et al*. Culture duration modulates collagen hydrolysate-induced tissue remodeling in chondrocyte-seeded agarose hydrogels. Ann Biomed Eng 2007; 35: 1914-23. http://dx.doi.org/10.1007/s10439-007-9373-z

[65] Sosnik A, Sefton MV. Methylation of poloxamine for enhanced cell adhesion. Biomacromolecules 2006; 7: 331-8. http://dx.doi.org/10.1021/bm050693h

[66] Lv Q, Hu K, Feng Q, Cui F. Fibroin/collagen hybrid hydrogels with crosslinking method: Preparation, properties, and cytocompatibility. J Biomed Mater Res A 2008; 84: 198-207. http://dx.doi.org/10.1002/jbm.a.31366

[67] Sosnik A, Sefton MV. Semi-synthetic collagen/poloxamine matrices for tissue engineering. Biomaterials 2005; 26: 7425-35. http://dx.doi.org/10.1016/j.biomaterials.2005.05.086

[68] Suri S, Schmidt CE. Photopatterned collagen-hyaluronic acid interpenetrating polymer network hydrogels. Acta Biomater 2009; 5: 2385-97. http://dx.doi.org/10.1016/j.actbio.2009.05.004

[69] Kundu J, Dewan M, Ghoshal S, Kundu SC. Mulberry non-engineered silk gland protein vis-a-vis silk cocoon protein engineered by silkworms as biomaterial matrices. J Mater Sci Mater Med 2008; 19: 2679-89. http://dx.doi.org/10.1007/s10856-008-3398-1

[70] Zhang XH, Reagan MR, Kaplan DL. Electrospun silk biomaterial scaffolds for regenerative medicine. Adv Drug Deliv Rev 2009; 61: 988-1006. http://dx.doi.org/10.1016/j.addr.2009.07.005

[71] She ZD, Jin CR, Huang Z, Zhang BF, Feng QL, Xu YX. Silk fibroin/chitosan scaffold: preparation, characterization, and culture with HepG2 cell. J Mater Sci Mater Med 2008; 19: 3545-53. http://dx.doi.org/10.1007/s10856-008-3526-y

[72] Mandal BB, Kapoor S, Kundu SC. Silk fibroin/polyacrylamide Semi-interpenetrating network hydrogels for controlled drug release. Biomaterials 2009; 30: 2826-36. http://dx.doi.org/10.1016/j.biomaterials.2009.01.040

[73] Rammensee S, Huemmerich D, Hermanson KD, Scheibel T, Bausch AR. Rheological characterization of hydrogels formed by recombinantly produced spider silk. App Physics A 2006; 82: 261-4. http://dx.doi.org/10.1007/s00339-005-3431-x

[74] Cheung HY, Lau KT, Tao XM, Hui D. A potential material for tissue engineering: Silkworm silk/PLA biocomposite. Compos Part B Eng 2008; 39: 1026-33. http://dx.doi.org/10.1016/j.compositesb.2007.11.009

[75] Chew SY, Hufnagel TC, Lim CT, Leong KW. Mechanical properties of single electrospun drug-encapsulated nanofibres. Nanotechnol 2006; 17: 3880-91. http://dx.doi.org/10.1088/0957-4484/17/15/045

[76] Chiono V, Ciardelli G, Vozzi G, *et al*. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/poly(epsilon-caprolactone) blends for tissue engineering applications in the form of hollow fibers. J Biomed Mater Res A 2008; 85: 938-53. http://dx.doi.org/10.1002/jbm.a.31513

[77] Chiono V, Vozzi G, D'Acunto M, *et al*. Characterisation of blends between poly(epsilon-caprolactone) and polysaccharides for tissue engineering applications. Mater Sci Eng C 2009; 29: 2174-87. http://dx.doi.org/10.1016/j.msec.2009.04.020

[78] Cho SW, Jeon O, Kim JE, *et al*. Preliminary experience with tissue engineering of a venous vascular patch by using bone marrow-derived cells and a hybrid biodegradable polymer scaffold. J Vasc Surg 2006; 44: 1329-40. http://dx.doi.org/10.1016/j.jvs.2006.07.032

[79] Del Gaudio C, Bianco A, Folin M, Baiguera S, Grigioni M. Structural characterization and cell response evaluation of electrospun PCL membranes: Micrometric *vs.* submicrometric fibers. J Biomed Mater Res A 2009; 89: 1028-39. http://dx.doi.org/10.1002/jbm.a.32048

[80] Duling RR, Dupaix RB, Katsube N, Lannutti J. Mechanical characterization of electrospun polycaprolactone (PCL): A potential scaffold for tissue engineering. J Biomech Eng 2008; 130: 011006. http://dx.doi.org/10.1115/1.2838033

[81] Fabbri P, Bondioli F, Messori M, Bartoli C, Dinucci D, Chiellini F. Porous scaffolds of polycaprolactone reinforced with *in situ* generated hydroxyapatite for bone tissue engineering. J Mater Sci Mater Med 2010; 21: 343-51. http://dx.doi.org/10.1007/s10856-009-3839-5

[82] Gaumer J, Prasad A, Lee D, Lannutti J. Structure-function relationships and source-to-ground distance in electrospun polycaprolactone. Acta Biomater 2009; 5: 1552-61. http://dx.doi.org/10.1016/j.actbio.2009.01.021

[83] Ghasemi-Mobarakeh L, Prabhakaran MP, Morshed M, Nasr-Esfahani MH, Ramakrishna S. Electrospun poly(epsilon-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering. Biomaterials 2008; 29: 4532-9. http://dx.doi.org/10.1016/j.biomaterials.2008.08.007

[84] Jung Y, Kim SH, You HJ, Kim YH, Min BG. Application of an elastic biodegradable poly(L-lactide-co-epsilon-caprolactone) scaffold for cartilage tissue regeneration. J Biomater Sci Polym Edition 2008; 19: 1073-85. http://dx.doi.org/10.1163/156856208784909336

[85] Kotela I, Podporska J, Soltysiak E, Konsztowicz KJ, Blazewicz M. Polymer nanocomposites for bone tissue substitutes. Ceram Int 2009; 35: 2475-80. http://dx.doi.org/10.1016/j.ceramint.2009.02.016

[86] Raghunath J, Georgiou G, Armitage D, *et al*. Degradation studies on biodegradable nanocomposite based on polycaprolactone/polycarbonate (80:20%) polyhedral oligomeric silsesquioxane. J Biomed Mater Res A 2009; 91: 834-44. http://dx.doi.org/10.1002/jbm.a.32335

[87] Vaquette C, Frochot C, Rahouadj R, Muller S, Wang X. Mechanical and biological characterization of a porous poly-L-lactic acid-co-epsilon-caprolactone scaffold for tissue engineering. Soft Mater 2008; 6: 25-33. http://dx.doi.org/10.1080/15394450801887109

[88] Venugopal J, Zhang YZ, Ramakrishna S. Fabrication of modified and functionalized polycaprolactone nanofibre scaffolds for vascular tissue engineering. Nanotechnol 2005; 16: 2138-42. http://dx.doi.org/10.1088/0957-4484/16/10/028

[89] Yeh CC, Li YT, Chiang PH, Huang CH, Wang YW, Chang HI. Characterizing microporous PCL matrices for application of tissue engineering. J Med Biol Eng 2009; 29: 92-7.

[90] Gomes ME, Azevedo HS, Moreira AR, Ella V, Kellomaki M, Reis RL. Starch-poly(epsilon-caprolactone) and starch-poly(lactic acid) fibre-mesh scaffolds for bone tissue engineering applications: structure, mechanical properties and degradation behaviour. J Tissue Eng Regen Med 2008; 2: 243-52. http://dx.doi.org/10.1002/term.89

[91] Gualandi C, White LJ, Chen L, *et al*. Scaffold for tissue engineering fabricated by non-isothermal supercritical carbon dioxide foaming of a highly crystalline polyester. Acta Biomater 2010; 6: 130-6. http://dx.doi.org/10.1016/j.actbio.2009.07.020

[92] Guarino V, Ambrosio L. The synergic effect of polylactide fiber and calcium phosphate particle reinforcement in poly epsilon-caprolactone-based composite scaffolds. Acta Biomater 2008; 4: 1778-87. http://dx.doi.org/10.1016/j.actbio.2008.05.013

[93] Kim SH, Kim BS. Effect of unintended pores on vascular scaffold fabrication. Tissue Eng Regen Med 2008; 5: 594-9.

[94] Lebourg M, Anton JS, Ribelles JLG. Porous membranes of PLLA-PCL blend for tissue engineering applications. Eur Polym J 2008; 44: 2207-18. http://dx.doi.org/10.1016/j.eurpolymj.2008.04.033

[95] Meretoja VV, Helminen AO, Korventausta JJ, Haapa-aho V, Seppala JV, Narhi TO. Crosslinked poly(epsilon-caprolactone/D,L-lactide)/bioactive glass composite scaffolds for bone tissue engineering. J Biomed Mater Res A 2006; 77: 261-8. http://dx.doi.org/10.1002/jbm.a.30630

[96] Russias J, Saiz E, Deville S, *et al*. Fabrication and *in vitro* characterization of three-dimensional organic/inorganic scaffolds by robocasting. J Biomed Mater Res A 2007; 83: 434-45. http://dx.doi.org/10.1002/jbm.a.31237

[97] Williams JM, Adewunmi A, Schek RM, *et al*. Bone tissue engineering using polycaprolactone scaffolds fabricated *via* selective laser sintering. Biomaterials 2005; 26: 4817-27. http://dx.doi.org/10.1016/j.biomaterials.2004.11.057

[98] Atzet S, Curtin S, Trinh P, Bryant S, Ratner B. Degradable Poly(2-hydroxyethyl methacrylate)-co-polycaprolactone Hydrogels for Tissue Engineering Scaffolds. Biomacromolecules 2008; 9: 3370-7. http://dx.doi.org/10.1021/bm800686h

[99] Baker BM, Gee AO, Metter RB, *et al*. The potential to improve cell infiltration in composite fiber-aligned electrospun scaffolds by the selective removal of sacrificial fibers. Biomaterials 2008; 29: 2348-58. http://dx.doi.org/10.1016/j.biomaterials.2008.01.032

[100] Causa F, Netti PA, Ambrosio L, *et al*. Poly-epsilon-caprolactone/hydroxyapatite composites for bone regeneration: *in vitro* characterization and human osteoblast response. J Biomed Mater Res A 2006; 76: 151-62. http://dx.doi.org/10.1002/jbm.a.30528

[101] Ang KC, Leong KF, Chua CK, Chandrasekaran M. Compressive properties and degradability of poly(epsilon-caprolatone)/hydroxyapatite composites under accelerated hydrolytic degradation. J Biomed Mater Res A 2007; 80: 655-60. http://dx.doi.org/10.1002/jbm.a.30996

[102] Mondrinos MJ, Dembzynski R, Lu L, *et al*. Porogen-based solid freeform fabrication of polycaprolactone-calcium

phosphate scaffolds for tissue engineering. Biomaterials 2006; 27: 4399-408.
http://dx.doi.org/10.1016/j.biomaterials.2006.03.049

[103] Fu SZ, Gun G, Gong CY, *et al*. Injectable biodegradable thermosensitive hydrogel composite for orthopedic tissue engineering. 1. preparation and characterization of nanohydroxyapatite/poly(ethylene glycol)-poly(epsilon-caprolactone)-poly(ethylene glycol) hydrogel nanocomposites. J Phys Chem B 2009; 113: 16518-25.
http://dx.doi.org/10.1021/jp907974d

[104] Zhao S, Lee J. Supramolecular hydrogels instantaneously formed by inclusion complexation between amphiphilic oligomers and alpha-cyclodextrins. Macromol Res 2009; 17: 156-62.
http://dx.doi.org/10.1007/BF03218672

[105] Cascone MG, Barbani N, Cristallini C, Giusti P, Ciardelli G, Lazzeri L. Bioartificial polymeric materials based on polysaccharides. J Biomater Sci Polym Edition 2001; 12: 267-81.
http://dx.doi.org/10.1163/156856201750180807

[106] Ma G, Yang D, Su D, Mu X, Kennedy JF, Nie J. Preparation and properties of water-soluble chitosan and polyvinyl alcohol blend films as potential bone tissue engineering matrix. Polym Adv Tech 2010; 21: 189-95.

[107] Pazos V, Mongrain R, Tardif JC. Polyvinyl alcohol cryogel: optimizing the parameters of cryogenic treatment using hyperelastic models. J Mech Behav Biomed Mater 2009; 2: 542-9.
http://dx.doi.org/10.1016/j.jmbbm.2009.01.003

[108] Lee SY, Pereira BP, Yusof N, *et al*. Unconfined compression properties of a porous poly(vinyl alcohol)-chitosan-based hydrogel after hydration. Acta Biomater 2009; 5: 1919-25.
http://dx.doi.org/10.1016/j.actbio.2009.02.014

[109] Brandl FP, Seitz AK, Teflmar JrKV, Blunk T, Göpferich AM. Enzymatically degradable poly(ethylene glycol) based hydrogels for adipose tissue engineering. Biomaterials 2010; 31: 3957-66.
http://dx.doi.org/10.1016/j.biomaterials.2010.01.128

[110] Patel PN, Smith CK, Jr. CWP. Rheological and recovery properties of poly(ethylene glycol) diacrylate hydrogels and human adipose tissue. J Biomed Mater Res A 2005; 73: 313-9.
http://dx.doi.org/10.1002/jbm.a.30291

[111] Lutolf MP, Hubbell JA. Synthesis and physicochemical characterization of end-linked poly(ethylene glycol)-co-peptide hydrogels formed by Michael-type addition. Biomacromolecules 2003; 4: 713-22.
http://dx.doi.org/10.1021/bm025744e

[112] Rice MA, Waters KR, Anseth KS. Ultrasound monitoring of cartilaginous matrix evolution in degradable PEG hydrogels. Acta Biomater 2009; 5: 152-61.
http://dx.doi.org/10.1016/j.actbio.2008.07.036

[113] Vermonden T, Fedorovich NE, van Geemen D, *et al*. Photopolymerized thermosensitive hydrogels: synthesis, degradation, and cytocompatibility. Biomacromolecules 2008; 9: 919-26.
http://dx.doi.org/10.1021/bm7013075

[114] Kavlock KD, Pechar TW, Hollinger JO, Guelcher SA, Goldstein AS. Synthesis and characterization of segmented poly(esterurethane urea) elastomers for bone tissue engineering. Acta Biomater 2007; 3: 475-84.
http://dx.doi.org/10.1016/j.actbio.2007.02.001

[115] Zhang CH, Wen XJ, Vyavahare NR, Boland T. Synthesis and characterization of biodegradable elastomeric polyurethane scaffolds fabricated by the inkjet technique. Biomaterials 2008; 29: 3781-91.
http://dx.doi.org/10.1016/j.biomaterials.2008.06.009

[116] Scott ON, Begley MR, Komaragiri U, Mackin TJ. Indentation of freestanding circular elastomer films using spherical indenters. Acta Mater 2004; 52: 4877-85.
http://dx.doi.org/10.1016/j.actamat.2004.06.043

[117] Ju B-F, Wan K-T, Liu K-K. Indentation of a square elastomeric thin film by a flat-ended cylindrical punch in the presence of long-range intersurface forces. J Appl Phys 2004; 96: 6159-63.
http://dx.doi.org/10.1063/1.1812822

[118] Liu KF, Van Landingham MR, Ovaert TC. Mechanical characterization of soft viscoelastic gels *via* indentation and optimization-based inverse finite element analysis. J Mech Behav Biomed Mater 2009; 2: 355-63.
http://dx.doi.org/10.1016/j.jmbbm.2008.12.001

[119] Lanza RP, Langer R, Vacanti J, editors. Principles of Tissue Engineering. 2nd ed. San Diego: Academic Press; 2000.

[120] Raghavan D, Kropp BP, Lin H-K, Zhang Y, Cowan R, Madihally SV. Physical characteristics of small intestinal submucosa scaffolds are location-dependent. J Biomed Mater Res A 2005; 73: 90-6.
http://dx.doi.org/10.1002/jbm.a.30268

[121] Fisher JP, Mikos AG, Bronzino JD, editors. Tissue Engineering. Boca Raton: CRC Press; 2007.

[122] Peppas NA, Merrill EW. Crosslinked poly(vinyl alcohol) hydrogels as swollen elastic networks. J Appl Polym Sci 1977; 21: 1763-70.
http://dx.doi.org/10.1002/app.1977.070210704

[123] Flory P. Principles of Polymer Chemistry. Ithaca, NY: Cornell University Press; 1953.

Received on 27-10-2015 Accepted on 16-12-2015 Published on 12-01-2016

DOI: http://dx.doi.org/10.6000/1929-5995.2015.04.04.1