Available online at www.sciencedirect.com

SciVerse ScienceDirect

Procedia Engineering 59 (2013) 117 – 125

Procedia Engineering

www.elsevier.com/locate/procedia

3rd International Conference on Tissue Engineering, ICTE2013

Role of Anisotropy in Tissue Engineering

Geoffrey R. Mitchell*, Ana Tojeira

Centre for Rapid and Sustainable Product Development – Polytechnic Institute of Leiria, *Centro Empresarial da Marinha Grande, Rua de Portugal – Zona Industrial,2430-028 Marinha Grande, Portugal*

Abstract

Tissue engineering is a highly interdisciplinary field that requires the integrated expertise of clinicians, cell biologists, engineers and material scientists, to make progress in the development and deployment of biological substitutes that restore, maintain, or improve tissue function. The purpose is to provide the opportunities for tissue regeneration and organ replacement. Key advances in biological materials especially in the area of stem cells; growth and differentiation factors generate realistic opportunities to create tissues in the laboratory using an engineered extracellular matrix or scaffold and biologically active molecules. The scaffold acts as an artificial extracellular matrix and it needs to mimic the chemical composition and physical architecture of natural extracellular matrix to facilitate cell adhesion, proliferation, differentiation and new tissue formation. In this contribution we review the role of the scaffold system in promoting cell adhesion, proliferation and differentiation with respect to the anisotropic nature of the scaffold system. We address both the anisotropy which may exist at a microscopic or mesoscopic scale, for example the shape of pores as well as the molecular level interactions which may arise in a scaffold containing a molecular organization with a preferred orientation which may have been induced during the processing procedures used to prepare the scaffold. Of course some approaches to the preparation of scaffolds systems are inherently anisotropic, for example the wide-spread utilization of meshes prepared by electrospinning. In other words although the overall scaffold is isotropic, the basic elements in terms of an electrospun fibre is highly anisotropic in terms of its external form and possibly in terms of its internal structure. By reviewing the possible advantages of the inclusion of anisotropic elements in the scaffold we add to the knowledge base which allows scaffolds design to be optimised for specific tissue growth.

Selection and peer-review under responsibility of the Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, Centro Empresarial da Marinha Grande.

*Keywords:*Tissue Engineering, Scaffold, Anisotropy, Morphology, Topography

---

* Corresponding author. Tel.: +351-244-569441; fax: +351-244-569444.
*E-mail address:*geoffrey.mitchell@ipleiria.pt

Selection and peer-review under responsibility of the Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, Centro Empresarial da Marinha Grande
doi:10.1016/j.proeng.2013.05.100

1. Introduction

Tissue engineered constructs (TEC's) can be design accordingly to "bottom-up" and "top-down" approaches. The "top-down" approach relies on the ability of cells to populate scaffolds and to recreate a suitable environment and proper vascularization through cell activity mechanisms aided by dynamic bioreactors (Urciolo et al. (2012)).

One important component of this strategy – the scaffold – carries out fundamental roles on the form, function, formation and fixation of this constructs on the site of the damaged tissue.

The tri-dimensional fillingin of the defect detected through imaging techniques (CT and MRI) is considered to be the *Form* of the construct (i.e., the overall shape of the biodegradable implant) whereas *Function* comes from the temporary mechanical support given by this structure to the growing tissue. This parameter is directly related to the scaffold design so that it avoids mechanical failure or resorption of the surrounding tissues. Tissue *formation* is highly influenced by the scaffold permeability and delivering of biologically active substances. The final characteristic of the scaffolds relates to the scaffold application on the site of the damaged tissue. The final product must be suited for ready implantation procedures where the surgeon attaches and fixates the scaffold to the neighboring tissues with swiftness and ease (Hollister (2009)).

Anisotropic features on scaffolds have been widely discussed in terms of scaffolds morphology and topography and the effects of such features in tissue viability.

2. Anisotropy

An isotropic structure is one which exhibits the same properties or structure when viewed from any direction. For example a sphere is isotropic; it has a single characteristic length which is the same in all directions. A rod has an anisotropic shape in which the characteristic length differs along its length and across its diameter. A collection of rods may have an anisotropic distribution with respect to a specific direction, for example the electrospun fibers shown in Fig. 1b.

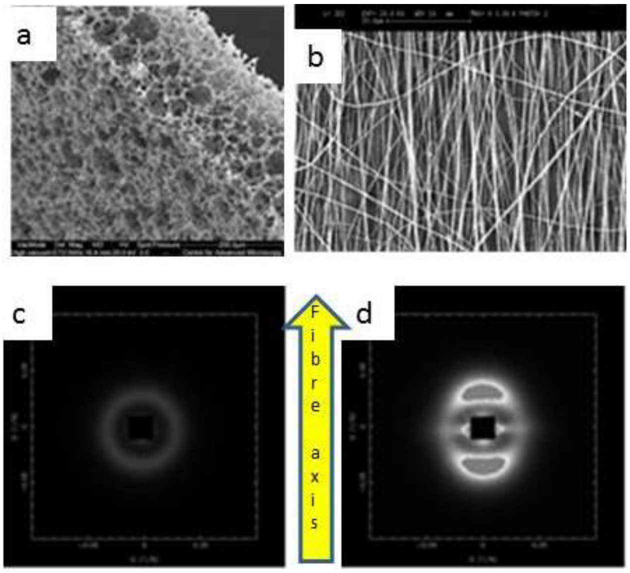

Fig. 1. (a) A scanning electron micrograph of the commercial scaffold material Alvetex® which is a porous cross-linked polystyrene membrane (Hayman et al. (2004)); b) Electrospun fibres prepared using an aqueous polyethyleneoxide solution together with a rotating collector; (c) Small-angle x-ray scattering pattern of an isotropic crystalline sample of poly(ε-caprolactone) showing the scattering from the crystalline lamellar; (d) small-angle x-ray scattering pattern of a highly anisotropic distribution of crystalline lamellar in a poly(e-caprolactone) templated using organic nano-particles (Wangsoub et al.(2008)).

Clearly the definition of anisotropy requires a specification of scale; for example the chemical bonds in a polymer chain may have an anisotropic angular distribution, those polymer chains may be aligned with the fiber axis, the polymer chains may be incorporated in to a chain folded lamellar crystal which have a preferred orientation as revealed by small-angle x-ray scattering as shown in Fig. 1d. This type of molecular anisotropy is clearly on a much smaller scale than the anisotropic shape of the electrospun fibers and its interaction with cells will take place on a scale different to the topology of the scaffold. The internal structure of the fibers may be defined by the processing characteristics as discussed by Edwards et al. (2010). This is likely to be a stronger feature of semi-crystalline polymers such as poly(ε-caprolactone) or poly(ethyleneoxide) as the crystallization process can be directed by the development of anisotropic nuclei during processing or through the inclusion of nanoparticles such as carbon nanotubes or grapheme nanoflakes.

The processes involved in the preparation of the scaffold will largely determine the nature of the anisotropy present. In many cases the angular distribution will be defined by a single angle with respect to the symmetry axis for example the fiber axis. In such cases the system may exhibit a rotational symmetry about the fiber axis and the level of anisotropy can be described using a series of amplitudes of spherical harmonics $<P_2n>$ (Mitchell and Windle (1988), Mitchell (2014)). Such information can be easily obtained from scattering or other experiments.

3. Fabrication techniques for anisotropic scaffolds

Scaffold fabrication interplays directly with its function in in vivo applications. Anisotropic scaffolds that mimic natively aligned tissues can be obtained via conventional and additive manufacturing techniques.

Solid Free-Form (SFF) fabrication is a layer-by-layer process capable to reproduce highly complex tri-dimensional scaffolds by computer aided-design (CAD) systems or through the converted medical imaging files (CT or MRI). These fabrication techniques allow scaffolds to be built with uniform and variable pores sizes as well as pores interconnectivity with accuracy and consistency. In addition, these techniques present a real advantage in not requiring toxic solvent or pore forming chemicals which may endanger patient health (de Mulder et al. (2009)).

On the other hand, conventional techniques are often less costly technologies able to create highly interconnected porous nets or micro-channels which present a foam-like or micro-channel morphologies.

3.1. Nozzle-based techniques

Fused Deposition Modeling (FDM) processes materials above their melting point temperature. The resulting slurry passes through a nozzle and is further deposited onto an elevating platform in the shape of a strand that cools down and solidifies. To form a tri-dimensional scaffold, each layer is deposited in a different configuration, i.e. at a different deposition angle, obtaining patterns such as 0º/90º or 0º/60º7120º. Fibre diameter, writing speed, extrusion rate and processing temperature are the main controllable parameters of these processes (de Mulder et al (2009)). Conversely, 3D Fibre Deposition (3DF) uses room temperature conditions to fabricate strands enabling this technology to produce cell-laden scaffolds. In both cases, the anisotropic morphology of scaffolds is directly affected by the directionally-dependent production of each layer.

Another scaffold fabrication concept firstly developed by Landers and Mulhaupt in 2000, aims to the production of scaffolds suitable for soft-tissue engineering applications. The tri-dimensional Bioplotting uses a 3-axis moving head to deposit material through filtered air pressure or a stepper motor-assisted system onto a stationary platform without demanding the use of high temperatures (Vaezi et al. (2012)). Khalil et al. (2005) conceived a multi-nozzle bioplotter suited for extruding biopolymer solution with living cells.

Electrospinning consists in the injection of a solution stored in a syringe thought a nozzle by applying an electric field producing fibers with a diameter in the nanometer scale (Fig. 2). Upon the application of an electrical field, the slurry at the tip of the nozzle becomes highly unstable due to rheological and electric properties of the fluid causing the solution to jet when the surface tension is overcome. During the fiber flight time, the solvent evaporates and a solid fiber is formed (Shin et al. (2012)).

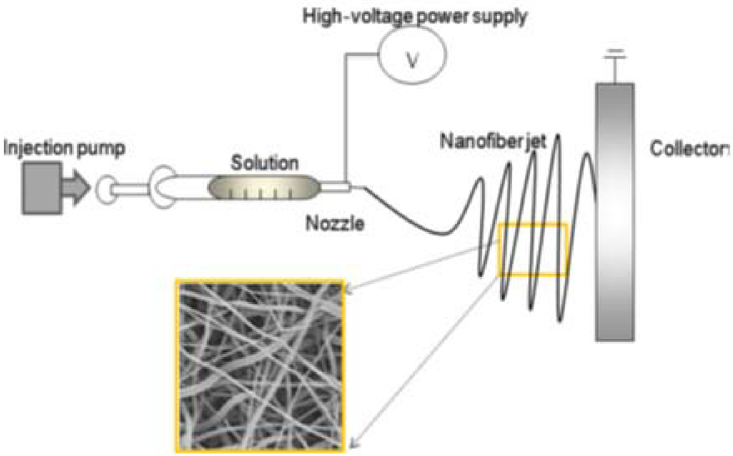

Fig.2. Electrospinning process with rotating mandrel for fiber alignment [9].

A Taylor's cone starts to form when a critical voltage is reached, propelling the fiber to draw a chaotic conical path in direction of the collector forming a highly porous net of overlapping nanofibers (Schiffman and Schauer (2008)). Several parameters from the electrospinning process such as voltage, electric current, injection rate, and environmental factors (e.g. temperature and humidity) may be controlled by the operator (Shin et al. (2012)).In order to obtain aligned fibers, the collector plate in the conventional electrospinning apparatus is substituted by a rotating mandrel at a defined speed, causing the meshes to present certain anisotropy (Sill and von Recum(2008)). The tangential velocity of the rotating collector must be slightly higher than the flight velocity of the electrospun fibers (~ m/s) in order to tension the fibers to facilitate the windup of the fibers with a common direction (Edwards et al. (2010)).

3.2. Printing-based techniques

Tri-dimensional printing (3DP) is an ink-based technology developed at the Massachusetts Institute of Technology. The scaffold is fabricated through deposition of droplets of binder material over a powder surface which induces the powder particle to stick together creating a solid part. The tri-dimensional construct is created by the superposition of bonded layers by subsequently lowering the powder bed and deposition of a new powder layer and repeating the binding process. Complex geometries and high interconnectivity of pores of scaffolds are possible to achieve with this technology(Vaezi et al. (2012)).

3.3. Printing-based techniques

Stereolithography is a layer-by-layer photosensitive resin polymerization using ultra-violet light (UV). Bártolo et al. (2009) indicated three different methods of irradiation which areoften applied to the curing of the scaffold material: mask-based, focusing beam or ink-jet-based processes. The first method creates an image generated by UV light cross patterned mask. The patterned light further irradiates the surface of the liquid polymer, curing and solidifying the scaffold layer. The following layers are generated in a similar way. The second method is considering being a direct writing technology, where the UV beam draws a path and polymerizes in situ the liquid polymer. At last in the ink-jet-based method, each layer is cured by UV irradiation immediately after jetting obtaining completely cured structures that require no post curing. This method is largely applied to tissue engineering due to high accuracy and precision of the photopolymerized scaffold.

3.4. Phase Separation techniques

Microcellular foams for Tissue Engineering have been largely produced by a method called Thermal Induced Phase Separation (TIPS). This approach produces isotropic scaffolds similar to that shown in Fig. 3. This process is based on thermal energy to obtain a multi-phase system. Martínez-Pérez et al. (2011) described that phase separation occurs when a homogenous solution separates into a polymer-rich phase and polymer-poor phase by exposure to an immiscible solvent which is extracted after the scaffold formation by lyophilization, or solution cooling below the bimodal solubility curve which represents the thermodynamic equilibrium of liquid-liquid de-mixing. In order to obtain anisotropic structures, a uniaxial thermal gradient is applied between two opposing sides of the matrix and insulation of the walls during the phase separation causes crystal formation longitudinally to the thermal gradient. This process creates micro-channels instead of the common ellipsoidal pores oriented in the same axis. The micro-channels diameter can be tuned by adjusting the thermal gradient and the polymer/solvent ratio. Some studies proved that high thermal gradient and high polymer concentration induces formation of micro-channels with small diameters. de Mulder et al. (2009) demonstrated that, as the same as the pore diameter, the microstructure of the micro-channels is also influenced by the solvent choice which can present microtubular or ladder-like morphologies.

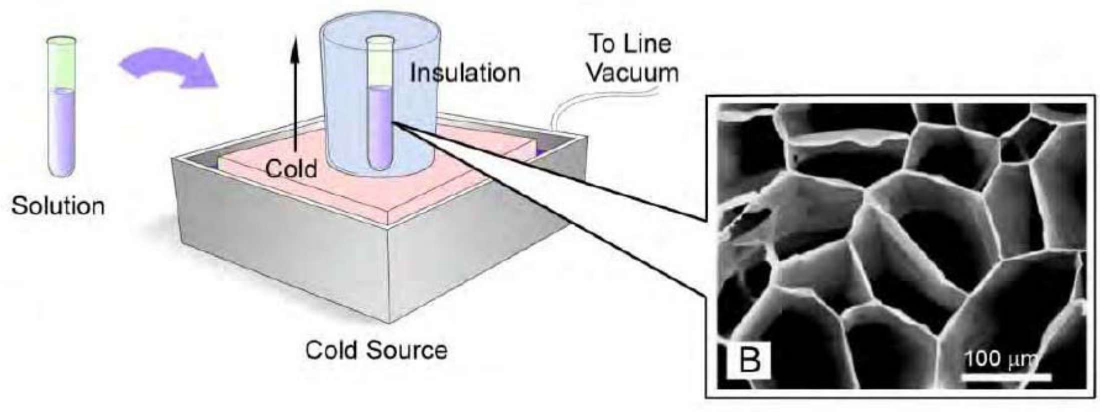

Fig.3. Thermally Induced Phase Separation adapted from Li et al. 2010.

Mathieu et al, (2004) fabricated porous scaffolds by using super-critical gas foaming techniques where pores are created by depressurization of a gas/polymer solution inducing the formation of bubbles. The CO_2 gas is generally dissolved on the polymer at a high pressure or a chemical blowing agent which yields gases production from its decomposition is added instead (Bártolo et al. (2009)).

4. Morphological anisotropy

4.1. Scaffold modulus

Nowadays, scaffold-based tissue engineering relies on the vision of producing constructs with mechanical properties similar to the host tissue capable to tune tissue regeneration at a cellular level (Freed et al. (2009)).

Conventionally-produced foam-like scaffolds are usually defined as highly interconnected networks with rounded, ellipsoidal or microtubular pores geometries. From a structural point of view, anisotropic foam scaffolds also present anisotropic mechanical properties. Mathieu et al. (2006) studied the compressive behavior of PLA/HA and PLA/β-TCP scaffolds fabricated via supercritical CO2 foaming, concluding that longitudinal compressive modulus was about 1.5 time the transverse modulus.

Relating to fibrous scaffolds, Ayres et al (2007) reported a study about the importance of alignment of collagen based fibers in mechanical properties of constructs. By adapting the Fourier transform (FFT), they measured the relative fiber alignment of electrospun fibers and correlated it with their fiber-orientation dependent tensile properties (longitudinal, perpendicular and transversally). The authors were able to establish quantitative criteria for determining the relative orientation of the fibers; and secondly, the tensile strength increased longitudinally to the fiber orientation at expense of the other orientations.

Li and co-workers (2010) used the 3DF technique to produce Ti6Al4V/methylcellulose scaffolds with deposition patterns of 0º/90º, 0º/45º/90º/135º and others. From this scaffold compressive tests were performed concluding that the compressive modulus in the Z-axis was considerably higher compared to the other patterns. However, the scaffold with 0º/90º deposition pattern showed enhanced compressive strength in the X- and Y-direction as well.

4.2. Cell-mediated scaffold contraction

Scaffold contraction is highly influenced by the contractile activity of cells within the constructs; therefore they can significantly alter the overall structure with mere cell migration and alignment mechanisms. Depending on the contractibility of cells, the structure of the scaffold starts to bend and to deform, altering the dimensions of the original scaffold (Corin and Gibson (2010)).

Caliariand Harley (2011) studied the contractibility effect of tendon cells seeded on collagen-glycosaminoglycans scaffold and cultured during a period of time of 14 days. Anisotropic scaffolds with larger pore sizes (243µm) presented lower scaffold contraction whereas isotropic scaffold with mean pore size of 87µm demonstrated greater percentage of contraction.

4.3. Scaffold permeability (vascularization)

The diffusion of nutrients and drugs whether as macromolecules or small dimension particles is an important issue regarding scaffold design. The transport of these molecules depends greatly on the diffusing molecule itself (size, charge, spatial configuration) but also in the scaffold design and volume fraction within the hosting tissue.Stylianopoulos et al.(2010) presented a work regarding random walk (steric interactions) and Stokesian dynamics (hydrodynamic interaction) simulations on the diffusion of macromolecules and particles in tri-dimensional constructs with different fiber alignment degrees in computer-generated stochastic models. From these calculations, they concluded that structure configuration doesn't affect the overall diffusion coefficient. However, directional components of the diffusion coefficient strongly depend on the fibers alignment degree, particle size, and fiber volume fraction.

4.4. Cell attachment

A primary contact of a cell with a scaffold is successfully noticed when integrin molecules located on the cell surface bonds with the scaffold surface. One important scaffold design parameter is the specific surface area resulting from the fabrication process of the construct once it represents the amount of surface available for cells to adhere and proliferate.Comparing isotropic and anisotropic scaffold geometries, Caliari et al. (2011) concluded that cell attachment and consequent proliferation was significantly more prominent in anisotropic scaffold.

4.5. Metabolic activity

As a neo-formed tissue, the metabolic activity is an import assessment parameter which informs about the health of the growing tissue. In the same study, anisotropic scaffold were found to support a higher metabolic activity of the tendon cells comparatively to the isotropic scaffolds. However, at the same time, the study conducted by Caliari et al. (2011) proved that metabolic activity was severely influence by the pore size of the scaffold.

4.6. Cell distribution (infiltration) and alignment

One of the most important issues regarding scaffold design relies on the infiltration of proliferating cells within the tri-dimensional construct. Besides supporting the initial cell attachment, induction of tissue formation from the outer edges of the scaffold to its core may be constraining to tissue viability.

Contrary to most native tissues which possess an optimized network of blood vessels and capillaries, *in vitro* and *in vivo* cultured TEC's commonly develop necrotic cores as consequence of limited cell penetration and decreased scaffold vascularization (Silva et al. (2006)).

Caliari et al. (2011) also studied the cell distribution within the isotropic and anisotropic scaffold by taking histological samples from the transversal and longitudinal cuts of the cell-seeded collagen-GAG scaffold after a14 day period of culture. From this essay, a remarkable cell penetration was observed in anisotropic scaffolds with larger pores. From this they concluded that highly aligned and porous scaffolds present a significant contact guidance cues which promotes cell motility within the network. In a similar study, Gonnerman, et al. (2012) reported HL-1 cardiomyocytes alignment achieved by contact guidance cues of provided by scaffold anisotropy and pore size and distribution of the collagen-GAG scaffold fabricated by freeze-drying.

5. Topographic anisotropy

Most types of cells are able to sense orientation, texture and physical properties of scaffolds. In a material perspective of the scaffold, topographic anisotropy of the constructs represents an important factor to cells motility, alignment and functions.

5.1. Cell response to topographical anisotropy

Considering isotropic scaffold morphology, the neo-formed tissues would gain an isotropic configuration further requiring a second step in the differentiation process after scaffold desorption in order to obtain an alignment similar to the native tissue (Davidenko et al. (2012)).

Such effect can be achieved, not altering the morphology of the scaffold, but assuring that the topographic and physical features of the scaffold at a nanoscale are suited for cell alignment and function. All materials contain topographic features; whether they are naturally imposed or altered by any surface modification process (Hoffman-Kim et al. (2012)).

Groove surfaces, aligned fibers, cell-inspired topographies and micro-channels are anisotropic features of scaffold surfaces. The alignment of cells due to topographic cues have been study as a feature to natively aligned tissue once the cells tend to minimize the distortion of their cytoskeletons and also that these pattern promote mechanical strength in the direction of the preferred orientation of the feature (Fig. 4) (Hoffman-Kim et al. (2012)).

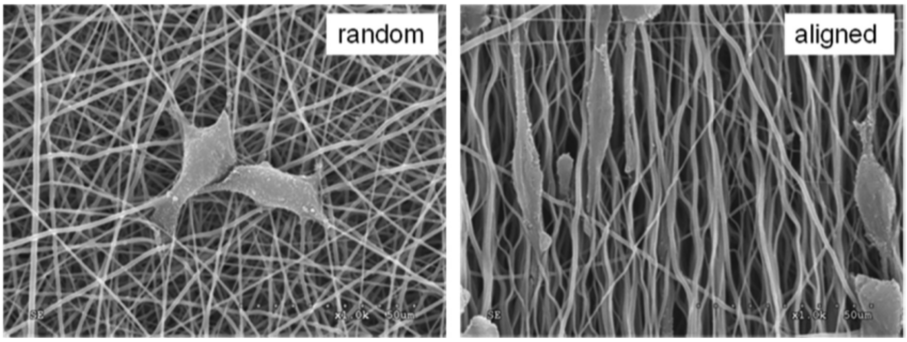

Fig.4. Alteration of cell morphology due to fiber alignment produced via Electrospinning (reproduced with permission from Journal of Tissue Engineering, Shin et al. (2012)).

6. Conclusions

The preparation of scaffolds may lead to a system made up of highly anisotropically shaped features or to the development of an anisotropic internal structure through the alignment of particles, molecules or crystals. This review of the roles of these features in determining the behavior of the scaffold has revealed that such features provide an additional methodology for tailoring the scaffold for specific applications

Acknowledgements

The authors would like to thank Matthew Spink and Dr. Imogen Smith of the University of Reading for permission to use the micrograph of the Alvetex® membrane which was a gift from Reinnervateplc and D.rSaeed Mohan of the University of Reading and Dr. Mohammed Al-Azab of the University of the United Arab Emirates for permission to use the micrograph of the polyethylene oxide electrospun fibers prior to publication. This work was supported by FCT.

References

Ayres, C. E., Bowlin, G.L., Pizinger, R., Taylor, L.T., Keen, C.A., Simpson, D.G., 2007. Incremental changes in anisotropy induce incremental changes in the material properties of electrospun scaffolds. ActaBiomaterialia 3, 651-661.

Bartolo, P.J., Chua, C.K., Almeida, H.A., Chou, S.M., Lim, A.S.C., 2009. Biomanufacturing for tissue engineering: Present and future trends. Virtual and Physical Prototyping 4, 203-216.

Caliari, S.R., Harley, B.C., 2011. The effect of anisotropic collagen-GAG scaffolds and growth factor supplementation on tendon cell recruitment, alignment, and metabolic activity.Biomaterials 32, 5330-.5340.

Corin, K.A., Gibson, L.J., 2010. Cell contraction forces in scaffolds with varying pore size and cell density. Biomaterials 31, 4835-4845.

Davidenko, N., Gibb, T., Schuster, C., Best, S.M., Campbell, J.J., Watson, C.J. and Cameron, R.E., 2012. Biomimetic collagen scaffolds with anisotropic pore architecture. ActaBiomaterialia 8, 667-676.

de Mulder, E.L.W., Buma, P., Hannik, G., 2009. Anisotropic Porous Biodegradable Scaffolds for Musculoskeletal Tissue Engineering. Materials 2, 1674-1696.

Edwards, M.D., Mitchell, G.R., Mohan, S.D., Olley, R.H., 2010.Development of orientation during electrospinning of fibres of poly(ε-caprolactone). European Polymer Journal 46, 1175-1183.

Freed, L.E., Engelmayr Jr., G.C., Borestein, J.T., Moutos, F.T., Guilak, F., 2009. Advanced Material Strategies for Tissue Engineering Scaffold. Advanced Materials 21, 3410-3418.

Gonnerman, E.A.; Kelkhoff, D.O., McGregor, L.M., Harley, B.A.C., 2012.The promotion of HL-1 cardio7.myocytes beating using anisotropic collagen-GAG scaffolds. Biomaterials 22, 8812-8821.

Hayman, M.W., Smith, K.H., Cameron, N.R., Przyborski, S.A., 2004. Enhanced neurite outgrowth by human neurons on solid three-dimensional scaffolds. Biochemical and Biophysical Research Communications 6, 483-488.

Hoffman-Kim, D., Mitchel, J.A., Bellamkonda, R.V., 2010. Topography, Cell Response,and Nerve Regeneration.Annual Review of Biomedical Engineering 12, 203-231.

Hollister, S.J., 2009. Scaffold Design and Manufacturing: From Concept to Clinic.Advanced Materials 21, 3330-3342.

Khalil, S., Nam, J., Sun, W., 2005. Multi-nozzle for construction of 3D biopolymer tissue scaffolds. Rapid Prototyping Journal 11, 9-17.

Li, P.J., Wijn, J.R., van Blitterswijk, C.A., de Groot, K., 2009. The effect of scaffold architecture on properties of direct 3D fiber deposition of porous Ti6Al4V for orthopedic implants.Journal of Biomedical Materials Research Part A 92, 33-42.

Martínez-Pérez, C.A., Olivas-Armendariz, I., Castro-Carmona, J.S., García-Casillas, P.E., 2011. Scaffold for Tissue Engineering Via Thermal Induced Phase Separation, in *"Advances in Regenerative Medicine"*In: Wislet-Gendebien S. (Ed.), InTech, Croatia, pp. 275.

Mathieu, L.M., Mueller, T.L., Bourban, P.-E., Pioletti, D.P., Muller, R., Manson, J.-A.E., 2006. Architecture and properties of anisotropic polymer composite scaffold for bone tissue engineering. Biomaterials 27, 905-916.

Mitchell, G.R., Windle, A.H., 1988. Orientation in Liquid Crystal Polymers in *"Developments in Crystalline Polymers"*. In:BassettD.C. (Ed.), Applied Science, pp. 115-175.

Mitchell G.R. 2014. Scattering Methods for Polymer Orientation Characterisation, Springer.

Schiffman, J., Schauer, C.L., 2008. A Review: Electrospinning of Biopolymer Nanofibers and their Applications. Polymer Reviews 48, 317-352.

Shin, S.-H., Purevdorj, O., Castano, O., Planell, J. A., Kim, H.-W., 2012. A short review: recent advances in electrospinning for bone tissue regeneration. Journal of Tissue Engineering3, 1-10.

Sill, T.J., von Recum, H.A., 2008. Electro-spinning: Applications in drug delivery and tissue engineering. Biomaterials 29, 1989-2006.

Silva, M.M.C.G., Cyster, L.A., Barry, J.J.A., Yang, X.B., Oreffo, R.O.C., Grant, D.M., Scotchford, C.A., Howdle, S.M., Shakesheff, K.M., Rose, F.R.A.J., 2006. The effect of anisotropic architecture on cell and tissue infiltration into tissue engineering scaffolds. Biomaterials 27, 5909-5917.

Stylianopoulos, T., Diop-Frimpong, B., Munn, L.L. and Jain, R.K., 2010. Diffusion Anisotropy in Collagen Gels and Tumors: The Effect of Fiber Network Orientation. Biophysical Journal 99, 3119-3128.

Urciuolo, F., Imparato, G., Guaccio, A., Mele, B., Netti, P.A., 2012. Novel strategies to engineering biological tissue in vitro, in *"Nanotechnology in Regenerative Medicine: Methods in Molecular Biology"*. In: Navarro, M. and Planell, J.A. (Eds.) Springer Science+Business Media, LLC, pp. 223.

Vaezi, M., Seitz, H., Yang, S., 2012. A review on 3D micro-additive manufacturing technologies. International Journal of Advanced Manufacturing Technology, 1-34.

Wangsoub, S., Davis, F.J., Mitchell, G.R., Olley, R.H., 2008. Enhance templating in the crystallization of Poly(ε-caprolactone) using 1,3:2,4-di(4-chlorobenzylidene) sorbitol. Macromolecular Rapid Communications 29, 1861-1865.