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2014

Rezende, R. A., Pereira, F. D. A. S., Noritomi, P. Y., Da Silva, J. V. L., Mironov, V., Baptista, L. S., et al. (2014). Third Strategy in Tissue Engineering: Tissue Spheroids Encaged into Microscaffolds. Proceedings of the 1st International Conference on Progress in Additive Manufacturing (Pro-AM 2014), 453-458.

<https://hdl.handle.net/10356/84270>

https://doi.org/10.3850/978-981-09-0446-3_107

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Downloaded on 26 Apr 2023 21:28:51 SGT

THIRD STRATEGY IN TISSUE ENGINEERING: TISSUE SPHEROIDS ENCAGED INTO MICROSCAFFOLDS

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ABSTRACT: Tissue engineering is a biomedical technology of artificial development of living three-dimensional human tissues and organs. Tissue engineering is based on two distinct premises. First more conventional approach uses solid biodegradable porous scaffolds as a temporal supporting framework for living cells attachment and sequential formation of three-dimensional tissue whereas second emerging approach is a solid scaffold-free directed tissue self-assembly with using tissue spheroids or microtissues as building blocks. In this paper novel hybrid approach or so-called third strategy in tissue engineering which combines advantages of first and second approaches is presented. The novel concept of lockyballs or tissue spheroids encaged into interlockable synthetic microcaffolds is described.

Keywords: tissue engineering, microcaffolds, tissue spheroids, two photon polymerization, photo-sensitive biomaterials, interlockable lockyballs.

INTRODUCTION

Two decades ago in 1993 influential breakthrough paper with short title **Tissue Engineering** was published in journal *Science* by pioneers of tissue engineering Joe Vacanti and Robert Langer (1993) which described a conceptual framework of this rapidly evolved direction in biomedical science and technology. The main element of their concept was the idea of scaffold as a temporal biodegradable structure for cell attachment and sequential formation of three-dimensional living human tissues.

The new solid scaffold-free approach in tissue engineering and regenerative medicine have been emerged which was called organ printing of bioprinting (Mironov et al., 2003; Mironov et al., 2008; Mironov et al., 2009). In essence it is a biomedical variant of well established rapid prototyping technology or additive layer by layer biofabrication of 3D tissue and organ constructs using tissue spheroids or microtissues as building blocks (Mironov et al., 2008; Mironov et al., 2009). This approach has been recognized as new paradigm (Willimas, 2009) and new perspective alternative research direction in tissue engineering and regenerative medicine (Derby, 2012). Organ printing is an integral part of more broad solid scaffold-free approach which was called directed tissue self-assembly (Mironov et al., 2008; Mironov et al., 2009; Gauvin and Khademhosseini, 2011; Kachouie et al., 2010). However, there is a growing consensus that a combination if these two strategies will be optimal, fruitful and could potentially lead to the development of novel third strategy in tissue engineering which will integrate advantages of both approaches (Kachouie et al., 2010; Norotte et al., 2009; Billiet et al., 2012). Finally, during the last years first attempts of formal combination of these two strategies have been published (Kim et al., 2010; Schon et al., 2012). The main argument against using method of directed tissue self-assembly and bioprinting using hydrogels was the absence of desirable material properties of tissue constructions biofabricated without using supporting solid scaffolds (Billiet et al., 2012). In this paper new technology platform will be presented, which logically integrate two distinct competitive strategies and create novel perspective third strategy in tissue engineering based on using tissue spheroids still capable to tissue fusion but encaged into solid synthetic interlockable microscaffolds with strong material properties.

ORIGIN CONCEPT OF LOCKYBALLS

The lockyballs concept or tissue spheroids encaged into interlockable solid biodegradable synthetic microscaffolds was born in Brazil (Rezende et al., 2012) (Figure 1). At first, it has been shown that increased rigidity of tissue spheroids after their directed induced tissue specific differentiation into cartilage (chondrospheres) or bone (osteospheres) tissue reduces their capacities to rapid tissue fusion (Hadju et al., 2010). Thus, for retention tissue spheroids together especially in the beginning the additional mechanism of their connection is becoming necessary. Secondly, immediately after orthotropic implantation of chondrospheres or osteospheres into the patient they undergo strong mechanical loading and compression which needs certain material properties which are absent in tissue engineered constructs biofabricated from tissue spheroids and embedded in soft hydrogel.

In order to withhold physiological mechanical loading tissue engineered constructs biofabricated from tissue spheroids must have certain material properties. The encaging tissue spheroid into solid biodegradable synthetic microscaffolds can provide desirable level of material properties. Thirdly, attempts to functionalize tissue spheroids or provide them with additional functions usually gives negative effects on viability of cell composing tissue spheroids and capacities of these cells to directed tissue differentiation (Bulte et al., 2004). Besides the obvious clinical success the use of tissue spheroids (chondrospheres) for treatment of cartilage tissue injuries developed by German biotech company Co.don has certain limitations and even potential risk. The chondrospheres attach to injured cartilage surface only by mechanism of surface tension forces and therefore patients must be immobilized immediately after implantation of tissue spheroids and later patients must avoid physiological compression and loading during several months. Finally, chondrospheres which are not well attached to patient cartilage tissue could be washed out and free floating chondrospheres in patient joint cavity could potentially lead to unwanted side effects and

undesirable complications (Wang et al., 2012). We assumed that encaging tissue spheroids into interlockable solid biodegradable synthetic microscaffolds will solve above described problems typical for method of directed tissue self-assembly with using tissue spheroids capable to tissue fusion.

Figure 1. (a) Original design of a lockyball and (b) lockyballs 3D printed by 2PP technique.

MICROSCAFFOLD FABRICATION TECHNOLOGIES

The two photon polymerization technology has been independently and practically simultaneously developed in Japan (Maruo et al., 1997), USA (Cumpston et al., 1999) and Russia (Borisov et al., 1998). The main difference of this method as compare with one photon polymerization methods of rapid prototyping or additive manufacturing such as stereolithography or selective laser sintering that it is a two photon polymerization method. The high concentration of energy in the point of intersection of two photons and using specially designed chemicals (photo-sensitizers) releasing free radicals during exposure to photons create necessary optimal conditions for rapid polymerization of photo-sensitive materials. Because employed photo-sensitive materials are transparent it is possible local polymerization not only from the surface as it is done in one photon polymerization technologies but in any point of three-dimensional volume or space filled with photo-sensitive materials. It is a unique method for fabrication complex geometric construction from material with nanolevel of resolution (Farsari and Chichkov, 2009). Finally, this method is based on principles of computer-aided design. Photo-sensitive material is polymerized layer by layer and it allows fabricating 3D microscaffolds of any level of complexity with high resolution. Two photon polymerization enables fabrication of microscaffold of précised geometry, shape and size according to preliminary created computer-aided design. Now broad library of biodegradable photo-sensitive biodegradable biomaterials have been developed and tested. Thus, it is important to indicate that method of two photon polymerization is one of most advanced optimal and rapidly progressing method of microscaffold biofabrication.

MATERIAL PROPERTIES OF MICROSCAFFOLDS

Estimation of material properties of microscaffolds is very important but not trivial task. The finite element analysis (FEA) (Figure 2c) demonstrates that more complex concentric geometry of lockyballs (Figure 2a) significantly enhances material properties of lockyballs. It has been shown during the comparative measurement using compression test performed with **Microsquisher** (Cellscale, Canada), that material properties of lockyballs with more complex internal concentric structure increased in 38 times (Figure 2b). Thus, microscaffolds indeed provide desirable rigidity

to tissue spheroids engaged into them. Microscaffolds in this case serve as a temporal support for bearing physiological mechanical loading and compression and at the same time provide time and conditions necessary for tissue fusion.

Figure 2. (a) Design of a concentric lockyball, (b) compression test with microsquisher and (c) view of a FEA simulation.

BIOFABRICATION OF TISSUE SPHEROIDS ENGAGED INTO MICROSCAFFOLDS

The incorporation of cells into microsccaffold or in essence biofabrication of tissue spheroids engaged into microsccaffolds or lockyballs is quite different from standard tissue engineered scaffold cellularization. The method of hanging drop works but with great level of variability in results. Sometimes cells formed tissue spheroids outside of microsccaffold, sometimes they form several tissue spheroids attached to each other. In short, this is also not an optimal method. Much more robust and reproducible is a method based on using micromolded non-adhesive agarose hydrogel developed by Jeff Morgan and commercialized by company Microtissues (<http://www.microtissues.com>). This technology guarantees the high level of control for cellularization and biofabrication of tissue spheroids and provides more reproducible results. It is critically important, however, to keep in mind ratio between diameter of recessions and external diameter of microsccaffold, between cellular size and size of pores in microsccaffold. Finally, 20-25% retraction of cell aggregate volume during tissue spheroid formation must be also considered. Thus, already existing methods allow reproducibly biofabricating tissue spheroids engaged into interlockable microsccaffolds.

PERSPECTIVES OF PRACTICAL APPLICATION OF TISSUE SPHEROIDS ENGAGED INTO MICROSCAFFOLDS IN TISSUE ENGINEERING

The impressive results on animal models with using cellularized self-assembled microsccaffolds have been recently demonstrated (Liu et al., 2011). Starting from 2006 German company **Co.don** which developed chondrospheres technology have performed already 5000 successful implantations of autologous chondrospheres to patients with cartilage injuries. Biopsy of tissue engineered cartilage after 18 months demonstrated development of practically normal hyaline cartilage in the zone of defect (Brochhausen et al., 2013). Tissue spheroids engaged into microsccaffolds will enable development of cost-effective tissue engineered treatment with using chondrospheres and osteospheres. Thus, cartilage and bone are most realistic areas for clinical translation technologies based on third strategy in tissue engineering.

CONCLUSIONS

Tissue spheroids encaged into micro scaffolds represents a new technology platform and perspective third strategy in tissue engineering based on combination of conventional solid scaffold based approach with emerging directed tissue self-assembly approach. The presented combined strategy enables rapid biofabrication of three-dimensional human tissues in vivo in operation room with sequential functional maturation of tissue constructs in patient organism. The interlockable micro scaffolds or lockyballs enhance material properties of 3D tissue constructs fabricated from tissue spheroids without compromising fusogenic capacities of tissue spheroids.

ACKNOWLEDGMENTS

This work has been sponsored by the São Paulo Research Foundation (FAPESP), The Brazilian Institute of Biofabrication (INCT-BIOFABRIS) and The National Council for Scientific and Technological Development (CNPq) through undergraduate program in Renato Archer Information Technology Center (CTI).

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