Zachodniopomorski Uniwersytet Technologiczny w Szczecinie

Department of Polymer and Biomaterials Science

Al. Piastów 45, 71-311 Szczecin, Poland

prof. dr hab. inż. Mirosława El Fray

tel: (+48) 91 499 48 28
fax: (+48) 91 499 40 98
Email: mirfray@zut.edu.pl

Review of doctoral dissertation of **Chiara Rinoldi**

titled **„Spun Fiber-based Scaffolds for Tendon Tissue Engineering"**

prepared at Faculty of Materials Science and Engineering,
Warsaw University of Technology
under supervision of Professor Wojciech Święszkowski, PhD, DSc
and co-supervision of Ewa Kijeńska-Gawrońska, PhD, MSc

Presented dissertation has been prepared as monograph containing 7 chapters on 177 pages with 5 tables, 38 figures and 215 references. I would like to present the scientific evaluation of the work in the following points:

1. Justification for tackling scientific problem
2. Novelty of research work
3. Analysis of research methodology
4. Conclusions

Justification for tackling scientific problem

Soft tissue reconstruction and regeneration is challenging especially for fibrous tissue with anisotropic properties. Tendons are an excellent example of nanofabrication, where biological material is built of parallel, nanometer scale (50 - 500 nm) fibrils, which assemble themselves

into a number of hierarchies of parallel collagen molecules. The collagen type I, which is dominant in tendons forms thick fibrils resistant to tension. The peak stress to which the tendon is subjected varies according to its anatomical site and the type of tendon (plantaris, deep digital flexor, gastrocnemius, to mention only a few). Progress in the understanding of the functional anatomy and mechanics of injured and deformed tendons and development of tissue engineering has been pivotal for repair and reconstruction of tendons. However, the most suitable combination of a material (polymer or composite) and processing technique to elaborate scaffold truly mimicking tendon structure and functionality is still remaining a big challenge. Therefore, the aim of the work was to develop and characterize novel fibre-based scaffolds for tendon tissue engineering. The research goal is therefore very timely and focused on two manufacturing techniques, namely electrospinning and wet-spinning to be used for polymer and hybrid (composite) scaffold fabrication.

Novelty of research work

Rapid development of tissue engineering which combines vital-avital components assembled into 3D architectures, called scaffolds, has accelerated research on artificial tissues, including tendons, significantly. Synthetic and natural polymers such as PLA, PGA, PCL, alginate, dextran and others, were already used to fabricate various scaffolding systems, mainly of fibrous architecture. Importantly, many different fabrication techniques involving knitting, braiding, weaving, wet-spinning and electrospinning led to successful outcome in term of fiber-based implants and scaffolds manufacturing. Considering natural, fibrous structure of tendon such approach seems to be most promising and the Candidate has selected wet-spinning and electrospinning as the most promising techniques to prepare cell-laden scaffolds. The concept of using micro- and nanosized multilayered fibres made of synthetic polymer and natural hydrogel modified with silica nanoparticle is novel and well addresses the requirements of suitable microenvironment for cell growth and orientation. To achieve the cell guidance by fibrous structures, dynamic mechanical stretching in presence of cells has been applied through custom-designed bioreactor. Overall, the novelty of research work is clearly presented in the light of existing state-of-the-art solutions described in Chapter I. Based on the gathered information, the overall objectives of the thesis and research hypotheses have been formulated as stated in Chapter II.

Analysis of research methodology

The results of research work are summarized in Chapters III-VI being reprints of three already published papers and one manuscript. In first paper published in *Journal of Materials Chemistry B*, poly(ε-caprolactone)/polyamide 6 (PCL/PA6) blends have been prepared for electrospinning. Additionally, polymer-nanosized silica nanocomposites have been prepared and used for processing. The derived bead-on-string fibrous structures and polymer blend fibres were characterized in term of surface chemical composition, morphology, wettability and degradation. Finally, cell studies have been performed with L929 fibroblasts to evaluate cell viability, proliferation, γ-tubulin and collagen I expression as well as glycosaminoglycan (GAG) production. The applied techniques and research methodology are adequate and confirmed the possibility of bead-on-string fibrous structures formation and demonstrate an advantageous role of silica nanobeads in surface stiffness and wettability enhancement, cell spreading, proliferation and extracellular components deposition. The target mechanical properties of polymer blends were comparable to supraspinatus tendon, however an addition of silica nanoparticles significantly decreased the ultimate tensile strength of PCL/PA6 blend and increased the elastic modulus.

The next step of research was the preparation of multilayered 3D scaffolds based on PCL/PA6 blends coated with thin layer of cell laden hydrogel. This work has been published in *ACS Biomaterials Science & Engineering* journal. The methacrylated gelatin (GelMA) and GelMA-alginate (Alg) blend has been converted into hydrogel upon UV curing (and crosslinking with Ca^{2+} ions when Alg has been used) taking place during deep coating of GelMA and/or GelMA-Alg on electrospun PCL/PA6 scaffolds. Biological studies have been extended to experiments with mesenchymal stem cells (MSC) as the most frequently used for tendon regeneration studies and with bone morphogenic proteins (BMP)s, where BMP-12 is known to trigger tenogenic pathway in stem cells. It has been demonstrated that custom-made testing device developed within this research in order to apply dynamic conditions mimicking native tissue loading-unloading patters significantly enhanced tenogenic differentiation of MSCs. It has been demonstrated that fabricated scaffolds at optimized concentration of BMP-12 showed synergistic effect of mechanical and biochemical stimulation resulting in superior cell adhesion, proliferation, alignment and differentiation. The array of testing method has been spanning mechanical tensile and compression tests, swelling and degradation profile experiments, and cell tests.

Additionally, real time polymerase chain reaction (RT-PCR) has been used to monitor relative gene expression of collagen type I, decorin, tenascin-C, scleraxis and tenomodulin.

It is not clear how many layers were applied to make a multilayer construct? The fabrication protocol indicate that the final construct is in fact a three-layer flat system with inner PCL/PA6 nanofibrous nonwoven mesh embedded in cell-laden hydrogel matrix.

It is also difficult to compare mechanical properties of electrospun fibres and hydrogel fibres as measurements were performed at different strain rates (5 mm/min *versus* 10 mm/min). How the difference in strain rate in general is affecting the mechanical tests?

The concept of multilayered scaffolds is better demonstrated in Chapter V discussing the research work published in *Advanced Healthcare Materials*. Combining co-axial extrusion with wet-spinning it was possible to obtain highly aligned gelatin methacryloyl-alginate yarns encapsulating hBM-MSCs. Wet-spun hydrogel fibres were collected onto a rotating drum to create densely packed bundles mimicking the architecture of tendon fascicles and its thickness. It was also demonstrated that fibrous structure and morphology of hydrogel yarns accelerated and enabled cell attachment and function, which has been more significant when mechanical and biochemical stimulation has been applied. Static stretching and presence of BMP-12 had positive effect on cell adhesion, alignment, proliferation and collagen I and III expression.

Final biomimetic arrangement of tendon structure has been proposed in Chapter VI (here presented as manuscript titled "*Biofabrication of 3D scaffolds formed from cell-laden hydrogel fibres layered on electrospun matrix loaded with BMP-12*" prepared for publication) by combining hydrogel fibres with electrospun polymeric mat to ensure suitable micro-environment for cell attachment and proliferation along with mechanical support to the construct. It has been demonstrated that enrichment of electrospun mat with BMP-12 has induced tenogenic differentiation followed by linear sustained release of the protein up to 30 days. In such a way, the research hypothesis has been fully addressed in exploiting the potential of 3D-multilayered scaffold composed of electrospun and hydrogel fibres to mimic the properties and anisotropic architecture of tendon tissue. However, the final mechanical properties assessed with DMTA are much lower compared to supraspinatus tendon which seemed to be the target tendon tissue as highlighted in Chapter III. The question arises how then such low mechanical properties will affect the tendon biofunctionality?

The performed research had solid basis on the results derived with the use of modern analytical, spectral, mechanical, biochemical/biological and biofabrication techniques. Importantly, interpretation of results is indicating scientific maturity of a Candidate. However,

it should be noted, that all published papers had multiple co-authors from different universities who also actively contributed to interpretation of the results and defined their roles.

Conclusions

In view of the results achieved, I consider that Chiara Rinoldi's doctoral thesis titled "Spun fiber-based scaffolds for tendon tissue engineering" submitted for the review corresponds to the requirements for doctoral thesis under the Act on Scientific Degrees and Scientific Title (Article 13 of the Act of 14 March 2003. Dz.U. 2003, No 65, item 595, as amended), therefore I recommend the dissertation to be accepted and Ms Chiara Rinoldi to be admitted to further stages of the doctoral thesis and to the public defence.

Mirosława El Fray

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