Fibroblast Growth on Nanofiber Material Matrices and Changes to Their Mechanical Properties

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***Abstract*—Polycaprolactone (PCL) and gelatin electrospun nanofiber matrices are very promising for heart valve tissue engineering. The main aim of this study was to test the growth capability of fibroblasts on these materials and to determine their effect on the mechanical properties of the tested materials.**

Matrices from PCL and gelatin were placed in standard 8-well culture plates and cultured for six weeks with COS-7 cells. Viability of cells was assessed by acridine orange and ethidium bromide staining. Mechanical properties were compared before and after culture with COS-7 using uniaxile tensile tests.

In tested conditions proportion of viable cells after 6 weeks of culture constituted ~90%, with no significant differences between materials and samples. Significant reduction in maximum stress was seen for PCL, from 0.49±0.01MPa to 0.37±0.03MPa (p<0.05). Deformability of gelatin increased from 87.2±13.8% to 157.1±15.2% (p<0.05) after 6 weeks in culture. Modulus of elasticity did not change significantly for both tested materials.

We conclude that both materials are suitable for tissue engineering purposes due to their biocompatibility, but the rather prominent changes in mechanical features of gelatin raise some doubts about its use as stand-alone scaffold material.

***Index Terms*—Fibroblasts, heart valve, nanofibers, tissue engineering**

I. Introduction

Heart valve tissue engineering is a rapidly evolving field with promises to offer a solution for heart valve replacement procedures, a solution which would be better than currently applied biological or mechanical heart valve prostheses. It gives us hope of creating heart valve prostheses – substitutes from living tissue capable of repair and possibly growth, which would eliminate the pitfalls of the current surgical prostheses.

Currently the treatment for end-stage valvular disease is surgical replacement with either a mechanical or bioprosthetic prosthesis. Mechanical prostheses offer excellent durability, but being a foreign non-physiological material in the bloodstream they cause increased risk of endocarditis as well as even more importantly risk of thrombosis, which calls for life-long anticoagulation with all the possible side-effects associated with it [1].

Biological prostheses are most often of xenogenic origin – porcine aortic valves or bioprostheses made from bovine pericardium [2]. Although bioprostheses are significantly less thrombogenic their durability is limited and the rate of their degradation is recipient age-dependant, making them more suitable for the patient older than 65 years and much less recommendable in younger patients due to a potential need for a repeat procedure in future [3], [4]

There have been many attempts to create a heart valve replacement with capabilities to grow and repair itself using tissue engineering techniques. With this approach patients own cells, isolated from blood, fatty tissue, bone marrow or other sources, are seeded on a premade – valve leaflet-shaped starter matrix, which must be able to support cell growth and cell-to-cell interactions required to form the structures typical to a heart valve leaflet. The two principal types of starter-matrices used are either of biological or synthetic origin, respectively; they can be either decellularized xenogenic or allogenic fixed valve leaflets or they can be made from biocompatible and biodegradable polymers. Decellularization has been applied with varying success in many studies [5], but in general it is a very tedious method with a very fragile balance between the requirement to remove all the cellular material, to prevent immunogenic reactions, and to maintain the extracellular matrix (ECM) structures relatively undamaged, for better and faster reseeding with patients cells and to provide a mechanically sufficient scaffold. An ideal synthetic matrix should be biocompatible, biodegradable and of course technically reproducible. Besides that the scaffold should also have a cell-friendly surface and similar mechanical properties to the native heart valves. To guarantee stability after implantation the rate of scaffold degradation shouldn't exceed that of new ECM fiber production. Keeping this in mind it is of special interest to stress that the ideal matrix must be not only mechanically strong enough to withstand the pressures after implantation but also deformable enough, because it has been demonstrated that ECM formation by valve interstitial cells is strongly dependant on the stiffness/deformability of the scaffold [6], [7]. Minimum time required for de-novo ECM formation is 6-8 up to 20 weeks which also would be the time a scaffold has to maintain its mechanical properties, before it can be replaced by the newly formed tissue [8].

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II. The Aim

Objective of our study was to evaluate two polymer nanofiber materials for use as a matrix for heart valve tissue engineering purposes by testing their biocompatibility for COS-7 cells in vitro and by evaluating their mechanical stability and rate of degradation by assessing their mechanical properties after a prolonged culture with these fibroblast-like cells.

III. Materials and Methods

Electrospun nanofiber materials were ordered custom-made by Elmarco s.r.o., (Liberec, Czech Republic) from gelatin type A with density of 5.7 g/m^2 and 0.1mm thickness and polycaprolactone (PCL) with a density of 15.7 g/m^2, thickness - 0.29mm.

Tested material samples were placed in standard 8-well culture plates and cultured for six weeks with COS-7 cells, originally derived from an African Green Monkey kidney fibroblast cell line (ATCC 1651 CRL). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) with 5% fetal calf serum at 37 ℃ in humidified 5% CO2 atmosphere. Culture media were changed twice a week. After six weeks of culture viability of the cells was assessed microscopically by acridine orange and ethidium bromide staining.

Mechanical properties were analyzed using an uni-axial universal testing machine Zwick/Roell BDO-FB0.5TS (Zwick GmbH & Co, Ulm, Germany) equipped with test Xpert software (see Fig.1). The tested nanofiber scaffolds were cut into 3.5 mm wide and 15 - 20 mm long specimens. The thickness of all specimens was measured using a cathetometer MK-6 (LOMO, Saint Petersburg, Russia) with a precision of ±0.01 mm. Data are presented as means ± standard deviation. Paired Student's t-test was used for statistical comparisons.

IV. Results

In tested conditions COS-7 cells were growing well on both nanofiber materials forming an uniform monolayer, see Fig. 2. Proportion of viable cells after 6 weeks of culture constituted 92.6±7.4% on PCL material and 88.1±5.7% on gelatin samples, with no significant differences between materials and samples.

TABLE I: Summary of Mechanical Properties of PCL and Gelatin Samples before and after Culture with Green Monkey Fibroblasts for 6 Weeks. Modulus of Elasticity (E), Ultimate Stress (Σ M) and Ultimate Strain (Σ X) Are Shown

	Max stress (Mpa)	Max strain (%)	Modulus of elasticity (Mpa)
PCL	0.49±0.01	34.7±4.0	2.1±0.1
PCL (6 w. COS-7)	0.37±0.03	28.5±1.0	2.2±0.1
Gelatin	0.43±0.09	87.2±13.8	0.73±0.17
Gelatin (6 w. COS-7)	0.45±0.08	157.1±15.2	0.62±0.16

Results of the mechanical properties testing of the two nanofiber materials before and after culture with COS-7 cells are summarized in Table I. Significant reduction in maximum stress was seen for PCL, from 0.49±0.01MPa to 0.37±0.03MPa (p<0.05), see Fig. 3a. On the other hand deformability of gelatin after 6 weeks cultivation with COS-7 increased from 87.2±13.8% to 157.1±15.2% (p<0.05), see Fig. 3b. Modulus of elasticity did not change significantly for both tested materials – 2.1±0.1MPa before and 2.2±0.1MPa after cultivation with the fibroblast cell line for PCL and 0.73±0.17MPa and 0.62±0.16MPa for gelatin respectively.

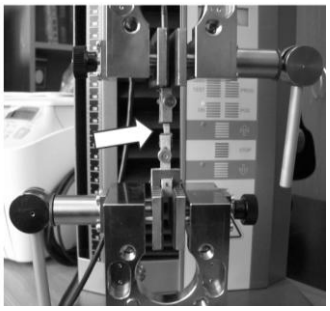

Fig. 1. The setup for uni-axial tensile tests with a universal testing machine Zwick/Roell BDO-FB0.5TS (Zwick GmbH & Co, Ulm, Germany) equipped with test Xpert software. Analyzed material sample marked with an arrow.

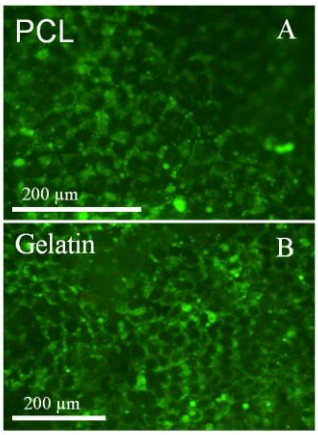

Fig. 2. Fluorescence microscopy images of COS-7 culture grown on PCL (A) and gelatin (B) samples for 6 weeks, acridine orange and ethidium bromide staining.

V. Discussion and Conclusions

Polymer scaffolds have been used for tissue engineering purposes with varying success for many years already [8]. Various polymers – natural and synthetic, their combinations and modified molecules have been tested for different tissue engineering tasks [9]-[11]. Many polymer scaffold types have been used previously for cardiac and cardiac valve tissue engineering [7], [8], [12]. Most interestingly there is recent data that one of our studied polymers – PCL containing several modifications and used in combination with polyethylene glycol can promote cardiomyogenic embryonic stem cell differentiation [13].

Both of the tested materials are widely studied and well known: one of them – polycaprolactone has been used as a material in surgery for decades with mechanical and biological properties, including biodegradation well described [14], [15]. Still to our best knowledge there are no published studies comparing mechanical properties of these two polymer nanofiber materials before and after prolonged cell culture.

PCL is an ideal candidate of tissue engineering scaffold for load bearing components, like cartilage and bone as well as heart valve tissue due to its slow degradation rate in vivo [14]. It has been shown that phagocytosis of PCL occurs after molecular weight of the polymer is reduced to 3000 or less by nonenzymatic hydrolysis of the ester linkages, which occurs after ~6 months in vivo [15]. Although our results show significant reduction of maximum stress of PCL matrices after 6 weeks of culture, this reduction of 25% is rather technically non-significant, because in the physiological conditions loading of the valve would never reach those tested and, as can be seen both in Table I and Fig. 3, modulus of elasticity (the slope of the graph) remains unchanged for the samples before and after culture.

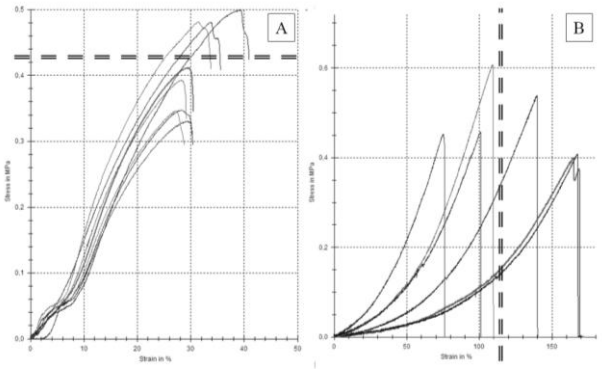

Fig. 3. Stress - strain curves of PCL and Gelatin from one of the experiments showing in a single graph both samples before and after culture with COS-7 cells. A: PCL samples – curves exceeding the double-dashed line correspond to those before culture, B: gelatin samples – curves to the left of the double-dashed line correspond to those before culture.

Gelatin although commercially widely used still remains a rather poorly understood substance manufactured from native collagen. Still it is rather very popular on the modern biomedical scene, being exploited as a drug and cell carrier/scaffold [10], [16]. It's mechanical properties and biodegradation both in vivo and in vitro has been extensively studied [10], [17]. In our study gelatin after 6 weeks in culture didn't lose its mechanical strength, but gained more deformability, which cannot be explained without ultrastructural studies. This in praxis could lead to problems in practical application of heart valve tissue engineered constructs with a scaffold made only from gelatin, because it could result in excess bulging and even prolapse of valve leaflets.

The main limitation of our study is that it has been carried out in an in vitro setting – namely data from a cell culture cannot be extrapolated to an in vivo situation with its highly dynamic environment in the circulatory system and exposure to high dynamic pressures. General conclusions about biocompatibility cannot be drawn from a study with COS-7 cells.

Our study has shown that both of the tested materials are suitable candidates for tissue engineering of heart valve substitutes. Both of them demonstrate good biocompatibility for COS-7 cells with ~90% of viable cells in the culture after 6 weeks, forming a monolayer which covers the entire sample.

The rather prominent changes in mechanical features of gelatin raise some doubts about its use as stand-alone scaffold material, judged by our findings it should be used together with other polymers, i.e. with PCL.

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