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Biodegradable membranes for neuronal growth and differentiation

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INTRODUCTION

Peripheral nerve injury is a worldwide problem: several hundred thousand cases of this injury occur each year in Europe and in the United States [1].

Fortunately, the peripheral nerve fibers, unlike those of the central nervous system, are able to regenerate after injury. Due to this property, surgeons are able to achieve a good functional recovery in patients who have suffered an injury to one or more nerve trunks through the realignment and suturing of the two stumps of the resected nerve. The resected fibers can thus regenerate along the graft and reach the distal nerve stump that will lead to their peripheral nerves.

To overcome disadvantages associated with autografts, xenografts and allografts, a wide range of materials have frequently been used for nerve regeneration. Recent research has been focused on the production of biodegradable artificial nerve guides which degrade within a reasonable period of time and manifest only mild foreign body reactions. In the last decade, with the rapid advances in biomaterial technology, several types of natural and synthetic biodegradable polymers have been reported as suitable for nerve regeneration [2-4]. To date, despite the significant number of developed functional biomaterials for nerve tissue engineering, the ideal physico-chemical compositions of such materials and the ideal surface structure and functionalisation have not yet been found.

In this context, semipermeable polymeric membranes with appropriate morphological, physico-chemical and transport properties are relevant to induce the neural regeneration [5-6]. Our investigation was focused on the development of novel biodegradable membrane systems aimed at providing an environment for nerve repair and a guidance for axonal migration, with suitable properties for supporting regeneration in term of cell adhesion and promotion of axonal outgrowth and differentiation. The final goal of this study is to develop highly membrane-mimicking peripheral nerve grafts for guided nerve tract regeneration.

METHODS

Membranes of chitosan (CHT), polycaprolactone (PCL), polyurethane (PU) and a biosynthetic blend between PCL and PU (PCL-PU) were prepared in flat configuration by phase inversion techniques. The biodegradable membranes were characterized. The morphological properties of all membranes and precisely mean pore size, pore size distribution and thickness were characterized by scanning electron microscope. The membrane wettability was characterized by water dynamic contact angle measurements. Degradation properties were investigated after enzymatic treatment. The mechanical properties were determined by measurements of ultimate tensile strength, Young modulus and elongation at break. We investigated the efficacy of these different membranes to promote adhesion and differentiation of neuronal cells. We employed as model cell system the human neuroblastoma cell line SHSY5Y, which is a well established system for studying neuronal differentiation. In particular, the capability of the developed biomaterials to enhance the differentiation of cells towards a neuronal phenotype was demonstrated by investigating in confocal laser microscopy and western blotting analysis, the expression pattern profile of specific neuronal proteins: axonal protein GAP 43, cytoskeletal protein distributed in the soma and in all neuronal processes beta-III- tubulin, with special

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attention to the most abundant integral membrane protein of synaptic vesicles, synaptophysin, for the detection of the synapse connections between the neurons.

RESULTS AND DISCUSSION

Biodegradable membranes prepared in this study displayed different morphological properties in terms of thickness, shape and sizes of the membrane pores, and also different physico-chemical, mechanical and biodegradation properties (Table 1).

Neuronal cells were able to adhere to and grow on the different substrates especially on PCL-PU membranes as demonstrated by the percentage of the area covered by cells and cell viability data.

MEMBRANE	**CHT**	**PCL**	**PU**	**PCL-PU**
Thickness [μm]	5 ± 0.9	8 ± 1	19 ± 1.9	11 ± 1.2
Mean Pore Diameter [μm]	0.026	0.022	0.082	-
E [MPa]	2.28	0.21	0.04	0.57
Dissolution [%]	100% 4 weeks	44% 6 months	35% 6 months	38% 6 months
WCA [°]	θ_{adv} 59 θ_{rec} 36	θ_{adv} 78 θ_{rec} 51	θ_{adv} 75 θ_{rec} 56	θ_{adv} 72 θ_{rec} 50

Table 1 Properties of biodegradable membranes: Young's modulus E; advancing θ_{adv} and receding θ_{rec} contact angle.

On membranes of synthetic biodegradable polymers (PCL, PU and PCL-PU) a neuron-like phenotype was developed that was characterized by spindle-like cell somata and extensive neuritis outgrowth. Interestingly, on these membranes the long and dense axons and dendrites which took compact arrangements, interweaved mutually to form neural networks which reached very complex density during the time especially on PCL-PU membranes after 6 days of culture (Fig.1).

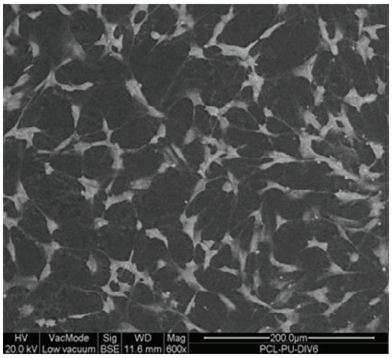

Fig.1. SEM's images of SHSY5Y cells after 6 days of culture on PCL-PU membranes

The *in vitro* reconstruction of neuronal network obtained in this work on the novel biodegradable membranes is a very important result which clearly indicates the cellular differentiation. Moreover, the expression pattern profile of GAP43, demonstrated that this axonal protein is strongly expressed by cells grown on PU and PCL-PU membranes, suggesting the ability of these substrates to support and promote neuronal outgrowth and synaptic plasticity in terms of axonal elongation.

The present work shows that neuronal cells respond to these novel biodegradable environment by changing their morphology and neurite outgrowth and regulating the expression and distribution of specific neuronal markers. The findings of this study confirmed that mechanical properties play an important role in driving cell-material interaction and provides evidences that it is one of the most important properties of substrate necessary for supporting neuronal survival and neurite growth influencing the cell's capacity to differentiate itself.

CONCLUSIONS

This work, for the first time, reports the use of PCL-PU blend membrane as a proposed material for nerve regeneration. This study provides evidence that the neural cell responses depend on the nature of the biodegradable polymer used to form the membranes as well as on the dissolution, hydrophilic and above all the mechanical membrane properties. PCL-PU membranes exhibit mechanical properties that improve the neurite outgrowth and the expression of specific neuronal markers.

Although PCL-PU membranes provide the most suitable conditions for promoting neuronal cell differentiation, the full set of results suggests that biodegradable membranes made of PCL and PU represent valuable supports to be used as *in vitro* systems that offer an adequate guide for nerve regeneration. Furthermore, our investigation suggests that blending could improve the mechanical properties of polymers and could also be a strategy for the development of an effective nerve guide.

Collectively, these findings represent a good statement for the realization of promising biomaterials and potential substitutes for the use of autologous nerve graft to repair short or medium length gaps in peripheral nerves.

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