

Selection of tuning PCL nanofiber non-wovens for muscle tissue engineering.

MN Giraud¹, D Keller¹, D Balazs², E Körner², T Humbert², H Tevaearai¹, G Fortunato²

¹ Swiss cardiovascular Center, Bern, Switzerland, ²EMPA, Saint Gallen, Switzerland.

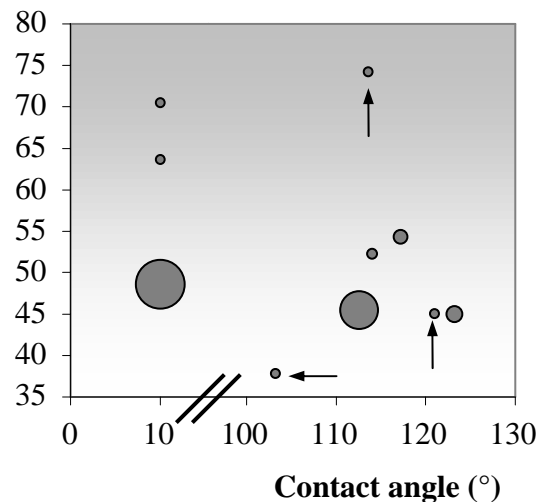
INTRODUCTION: Progress in tissue engineering is conditioned by the creation of a suitable environment in which cells can grow and organize themselves in a functional way. Electrospinning offer the capability to design nano-fibrous scaffolds in the form of nonwoven structure that can biomimetic ECM found in the native tissue¹. We addressed the role of nano- and micron-sized fiber structures in muscle cell proliferation, guidance and differentiation.

METHODS: The nano- and micron-sized non-wovens were prepared by an electrospinning procedure. Therefore, the biocompatible polymer polycaprolactone (PCL) was dissolved in appropriate solvents and spun by applying a high voltage on a needle tip. Partial parallelisation of the nanofibers was obtained by using electrostatic lens systems and a fast rotating drum. Selected fiber patches were coated with a nitrogen-functionalized amorphous hydrocarbon coating (a-C:H:N), by performing RF plasma deposition using a gaseous mixture of ammonia and ethylene. Myoblasts cell lines (C2C12) and primary myoblast cell culture were seeded on the surface of biomaterials and cultured for up to 2 weeks in growth and differentiation media. We used MTT assay for cell proliferation and immunostaining for cell differentiation.

RESULTS: The chemical and instrumental parameters used for the electrospinning procedure revealed a deep influence with respect to the PCL fiber diameters. Fibers with diameters from 100 nm to 2500 nm could be obtained. Main influencing parameters were the composition of the solvents used and the applied voltage.

Considering the factors such as fibers size, orientation (random or aligned) and wettability (contact angle) that may influence cell behaviors, we constructed a screening experiment to determine the optimal condition for muscle cell proliferation. The highest cell densities were obtained with aligned, 100 nm, hydrophobic PCL fibers and with 100 nm, randomly oriented, hydrophilic PCL fibers (Figure 1). Surface coating by plasma treatment of the biomaterials with the a-C:H:N provided an optimal cell adhesion that was increased by at least 20% with respect to non-coated patches. Interestingly, cell presented a specific

differentiation response with elongated and parallel myotubes (Figure 2).



DISCUSSION & CONCLUSIONS: Our results

Fig 1: Cell proliferation in response to electrospun PCL nano-fibrous scaffolds properties. Bubbles area represents fiber size from 100 to 2500 nm. Aligned fibers are indicated by arrow.

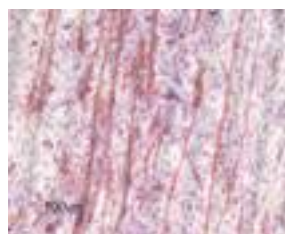


Fig.2. Aligned and elongated myotubes obtained from myoblast primary cell culture on a-C:H:N coated PCL (desmin immunostaining).

provide evidence that optimization of bioartificial tissue construct using nanofibers is challenged by biomaterials properties such as wettability, alignment and size of the fibers as well as specific coating. In addition parameters such as elasticity and porosity have to be considered for cell differentiation and the construction of a 3 dimensional bioartificial tissue.

REFERENCES:

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