Hydrolysis, Plasma and Aminolysis: how a surface activation method affects gelatin attachment to PLCL nanofibres

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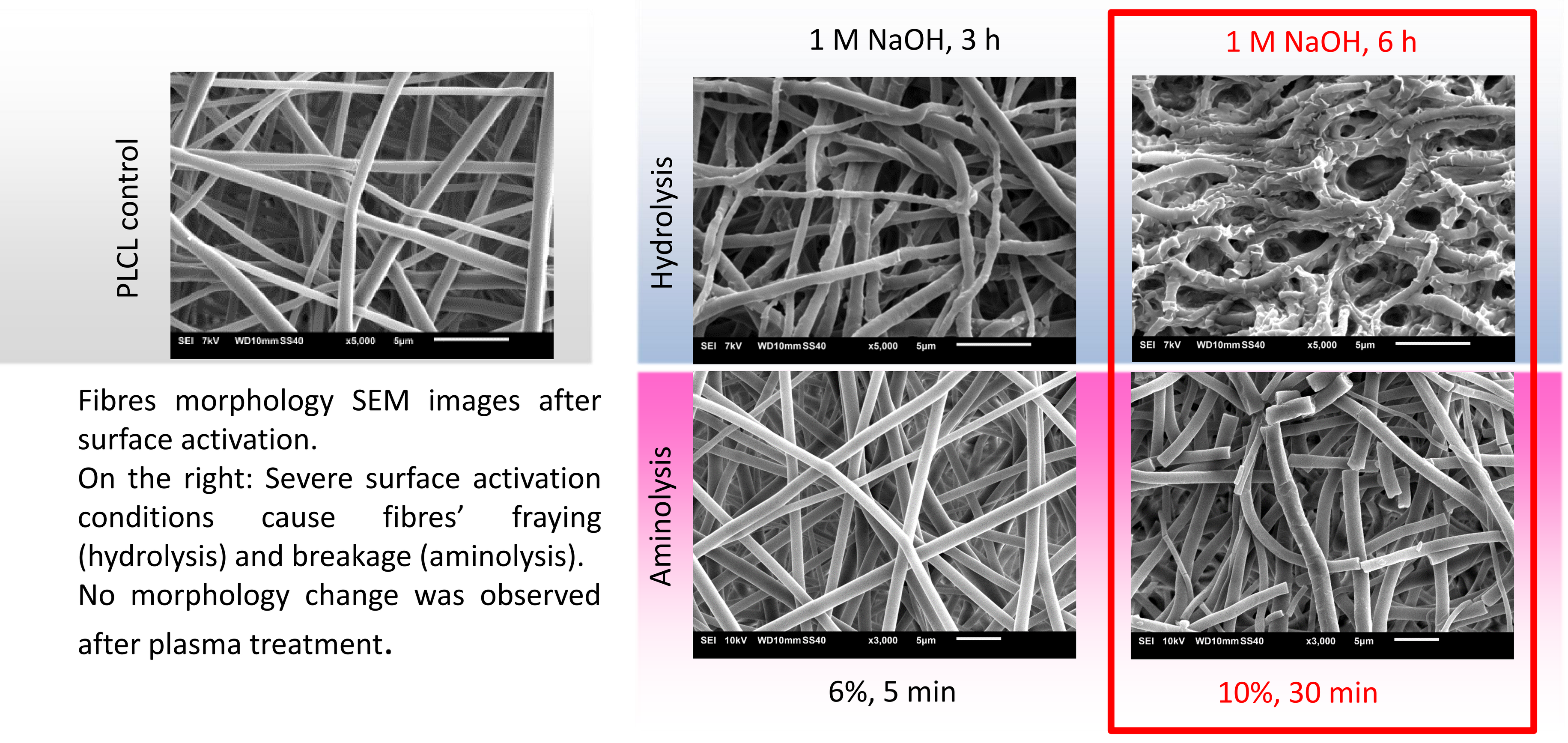
INTRODUCTION

Although aliphatic polyesters have many characteristics that make them widely used in tissue engineering they still are hydrophobic and lack bioactivity. Surface modification promises a solution to these problems.

Gelatin presence on a material's surface has been shown to have a beneficial effect on mammalian cells' proliferation and spreading [1]. Immobilization of gelatin through crosslinking to the surface of electrospun nanofibres made of aliphatic polyesters has been studied in our laboratory in the past years with the use of aminolysis as a surface activation technique [2]. In this work a series of experiments were performed on poly(L-lactide-co-ε-caprolactone) 70:30 (PLCL) nanofibres in order to compare three surface activation methods - **hydrolysis**, **cold oxygen plasma** treatment and **aminolysis**, which were used as a first step in a process of gelatin immobilization.

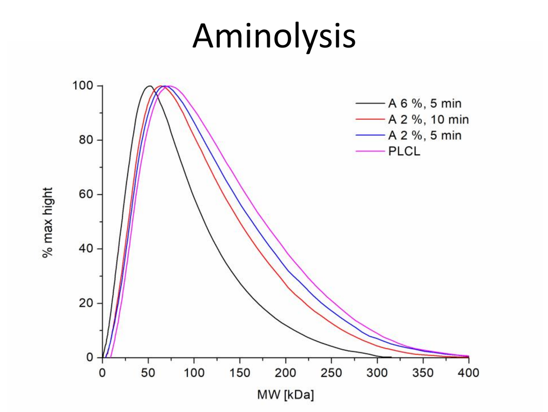
RESULTS

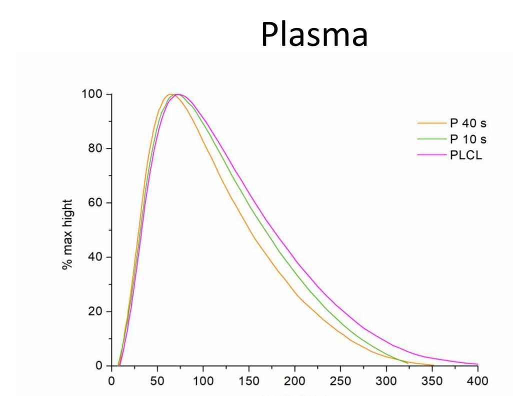
SCANNING ELECTRON MICROSCOPE (SEM) - morphology

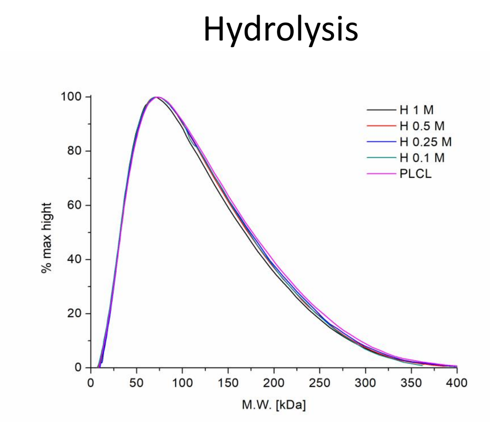


Fibres morphology SEM images after surface activation.
On the right: Severe surface activation conditions cause fibres' fraying (hydrolysis) and breakage (aminolysis).
No morphology change was observed after plasma treatment.

GEL PERMEATION CHROMATOGRAPHY – molecular weight

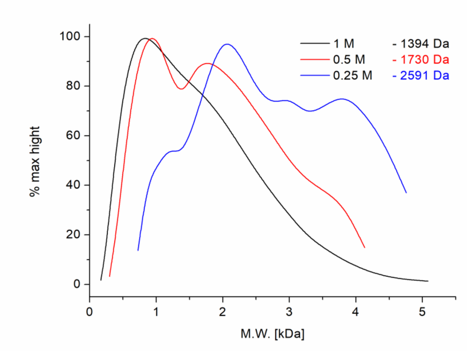






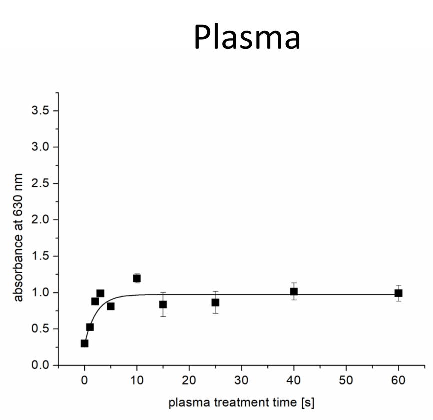
Surface activation method	M_w [kDa]	% M_{wPLCL} [%]	PDI
PLCL	58.7	100 %	1.50
A 2%, 5 min	49.8	85 %	1.63
A 2%, 10 min	47.8	81 %	1.59
A 6%, 5 min	30.6	52 %	1.91
P 10 s	54.5	93 %	1.51
P 40 s	52.5	89 %	1.48
H 1 M	57.7	98 %	1.48
H 0.5 M	57.3	97 %	1.50
H 0.25 M	59.6	101 %	1.47
H 0.1 M	56.4	96 %	1.51

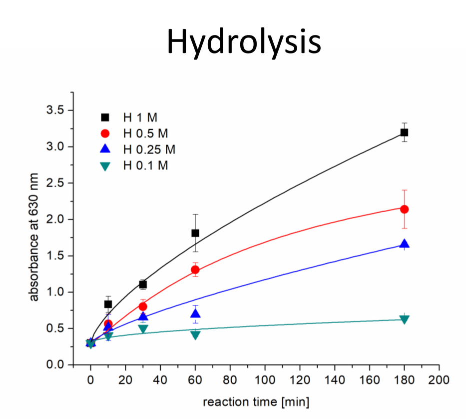
Molecular weight distribution graphs.
On the left: Data in the table shows mass average molar mass (M_w) values of the samples after surface activation and their comparison to the control PLCL sample.
On the right: A new peak for a very low molecular weight has appeared for the samples hydrolized with 1 - 0.25 M NaOH.



TOLUIDINE BLUE-O TEST – COOH group detection

Graphs presenting TBO test results obtained via UV-VIS spectrophotometry showing an increase in the COOH group amount on the surface of materials treated with cold oxygen plasma (left) and different concentrations of NaOH solutions (right) in a function of reaction time.
0 on the X axis represents PLCL control sample.





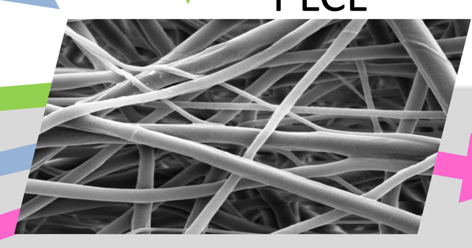
SURFACE ACTIVATION & GELATIN ATTACHMENT

Alkaline hydrolysis
- 1 – 0.1 M NaOH
- 3 h
- + COOH

Cold oxygen plasma
- 10, 40 s
- + COOH

Aminolysis
- 2 – 6 % diamine
- 5 – 10 min
- + NH2

PLCL



EDC/NHS
- 1h,
- 0.23%/0.12%
- COOH + NH2

GTA
- 2.5 h
- 1%
- NH2+NH2

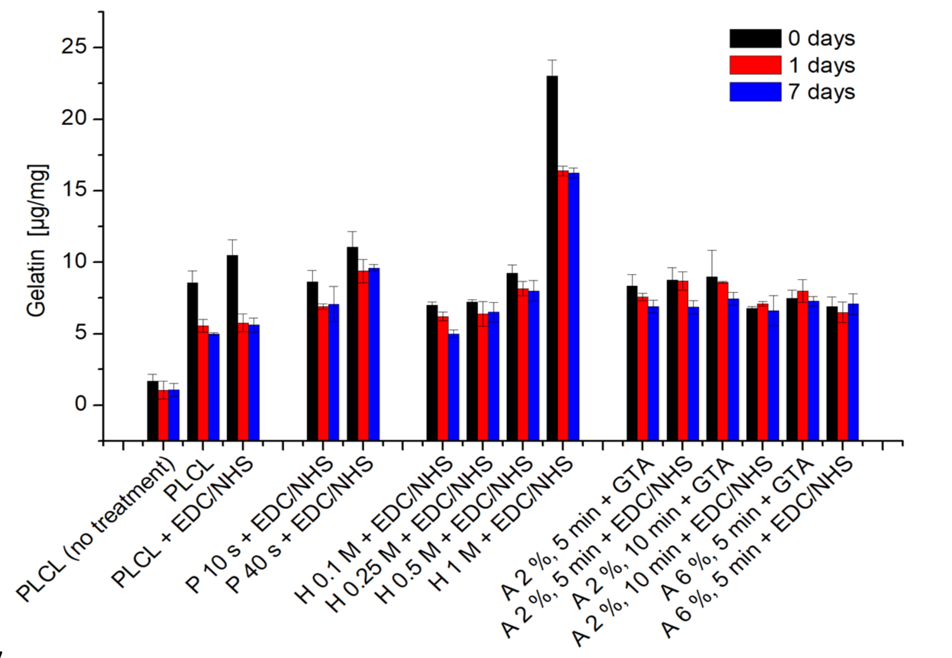
Control
- PLCL
- PLCL+ EDC/NHS

Gelatin attachment
- 0.02%
- 24 h
- 37°C

Attachment verification
- 1, 7 days
- 37°C, PBS, stirring

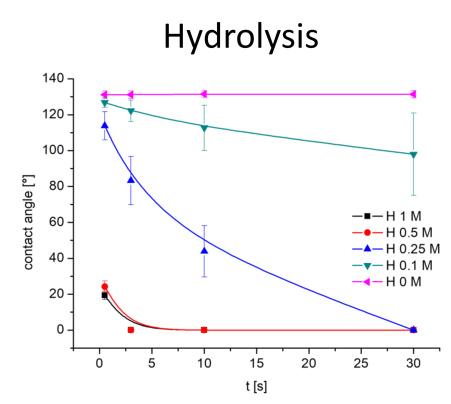
BICINCHONINIC ACID ASSAY (BCA) – protein attachment

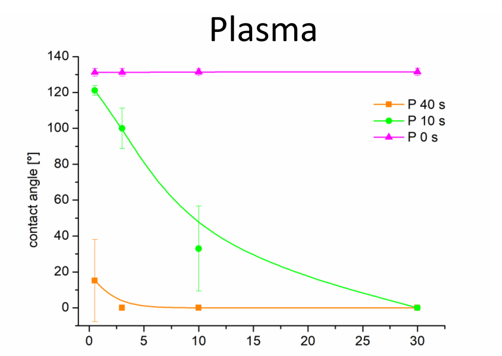
The comparison of BCA results shows protein content of PLCL nonwoven samples after different routes of gelatin immobilisation (0 days) and after attachment verification experiment consisting of immersion in PBS in 37°C for 1 and 7 days.
Control samples: Untreated PLCL, PLCL and PLCL+EDC/NHS with only gelatin physical adsorption.



WATER CONTACT ANGLE - hydrophilicity

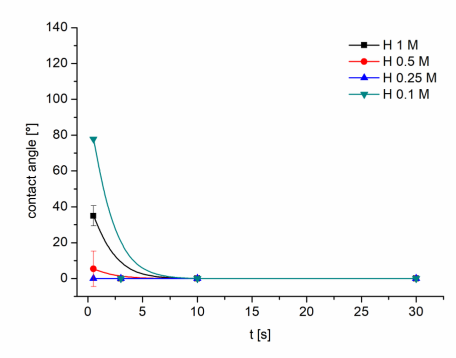
After surface activation



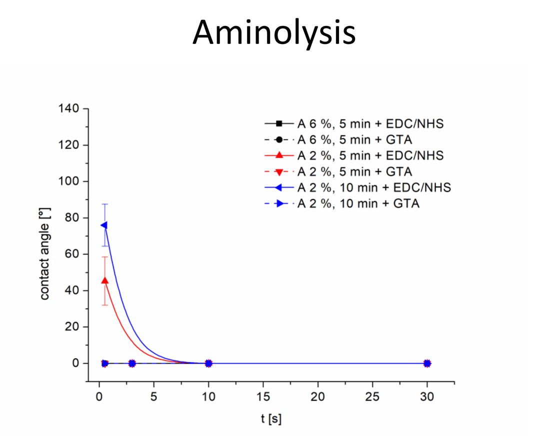


Up: Graphs showing a decrease in water contact angle due to surface activation with NaOH and plasma in function of a drop contact time with the material (0.2 - 30 s).
No change was observed for samples after aminolysis (PLCL≈131,43°).

After 7 days in PBS, 37°C



Down: Further decrease observed for all types of the samples due to immobilized gelatin present on the fibres' surface after attachment verifcation experiment. Plasma treated materials achieved 0° result for each measurement.



CONCLUSIONS

- All of the tested methods were successful in gelatin immobilization as well maintaining its attachment after PBS immersion test compared to the control.
- Apart from the samples treated with diamine the rest gained hydrophilicity after surface activation. After immersion in PBS all samples achieved 0°, what means that gelatin immobilisation successfully makes PLCL nonwoven hydrophilic.
- GPC showed that each activation method affected the molecular weight of the polymer differently with substential mass decrease of the samples treated with plasma and diamine. The smallest change was observed for the hydrolyzed samples. The observed here low molecular fraction peak, together with SEM imaging showing frayed fibres, indicate degradation taking place mostly on the fibre surface.
- The samples after both hydrolysis and plasma treatment showed elevated levels of COOH- groups, but between the two methods hydrolysis resulted in up to 3 more times higher values.
- The decision of which method is the most suitable should be based on a specific function that the final scaffold material is expected to fulfill and the properties it should have.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Dulnik, J, Kołbuk, D, Denis, P, Sajkiewicz, P 2018, 'The effect of a solvent on cellular response to PCL/gelatin and PCL/collagen electrospun nanofibres', *European Polymer Journal*, 104, 147-156
[2] Jeznach, O, Kołbuk, D, Sajkiewicz, P 2019, 'Aminolysis of various aliphatic polyesters in a form of nanofibers and films', *Polymers*, 11 (10), 1669