Biodegradation of bicomponent PCL/Gelatin nanofibres electrospun from alternative solvent system. Structure and properties analysis.

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ABSTRACT

Bicomponent polycaprolactone/gelatin nanofibers were formed by electrospinning as previously described [1] using a novel polymer – solvent system with solvents being alternative to the commonly used toxic solvents like fluorinated alcohols.

PCL/Gelatin nanofibres were electrospun from the mixture of acetic acid (AA) with formic acid (FA) (90:10) and from hexafluoroisopropanol (HFIP), that was used as reference solvent. PCL/Gelatin nanofibres with polymers w/w ratios 9:1, 8:2 and 7:3, underwent biodegradation in PBS solution at 37°C. After different times, ranging from 1 to 90 days, they were rinsed in demineralized water and dried. Weight loss and FTIR tests were performed to assess the kinetics of gelatin leaching, while SEM imaging and hydrophobicity tests to show its depletion from the surface. DSC measurements were carried out to examine changes in fibres' internal structure and uniaxial tensile testing to compare their mechanical properties.

Morphology of PCL/Gt fibers obtained from AA/FA is similar to that obtained from HFIP. Despite similar morphology, the internal structure of nanofibers formed from alternative solvents is different, reflecting the emulsive nature of PCL/gelatin mixture in AA/FA solvents contrary to clear, transparent solutions in HFIP. This apparent difference affects strongly the kinetics of leaching of gelatin from bicomponent fibres and thus how their mechanical and bioactive properties are changing in time after placing in living organism.

There is substantial difference in kinetics of gelatin leaching depending on solvent used. Mass measurements show much faster gelatin degradation in nanofibres electrospun from AA/FA than from HFIP. For instance, for PCL/Gt 7:3 samples, gelatin content loss is 85% for AA/FA and 68% for HFIP after 90 days. Moreover, irrespective of the solvent used, the degradation rate increases with initial content of gelatin and is the highest in the first 24 hours: 27% for AA/FA 9:1 and 67% for 7:3 and 13% and 32% for HFIP respectively. The observed changes can be explained by nonuniform distribution of gelatin within fibres spun form AA/FA due to emulsive character of solution. Comparison of SEM images reveals linear groove-like sites remaining after gelatin leaching on a surface of fibres spun from AA/FA solvent. Contrary to this, fibres spun from HFIP remain smooth which can be attributed to molecular dispersion of both components.

ACKNOWLEDGEMENT

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REFERENCES

[1] P. Denis, J. Dulnik, P. Sajkiewicz "Electrospinning and Structure of Bicomponent Polycaprolactone-Gelatin Nanofibers Obtained Using Alternative Solvent System" Journal of Polymeric Materials and Polymeric Biomaterials 64, 354–364 (2015)

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INTRODUCTION

The Polymer Physics Laboratory IPPT PAN has optimized the process of electrospinning of PCL/gelatin nanofibers based on the use of non-toxic solvents: acetic acid and formic acid [1] that are an alternative to perfluorinated alcohols. The disadvantage of this solvent system is the fact that polymer solution becomes visibly emulsive (Fig. 1), which is not observed in solutions from commonly used solvents.

The aim of this work was to investigate whether the solvent used in electrospinning influences the kinetics of gelatin leaching from PCL/gelatin nanofibers and how the properties of electrospun material are affected by this process.

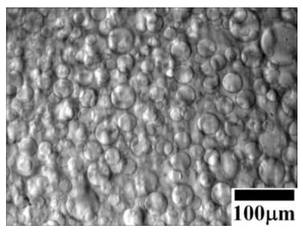


Fig. 1 Solution of PCL/Gt blend in AA/FA. Optical microscope.

EXPERIMENTAL

Polymers: Polycaprolactone (PCL) (Mn = 80 kD), gelatin (Gt) from porcine skin Type A (gel strength ~ 300 g bloom).

Solvents: 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), a mixture of acetic acid (AA, glacial pure 99,5%) and formic acid (FA, pure 98-100%) in 9:1 ratio.

Solutions: Pure PCL (PA, PH) and PCL/Gt blends in ratios 9:1, 8:2 and 7:3 in HFIP (PGH) and AA/FA (PGA) in 5% and 15% concentrations respectively.

Method: Electrospinning on rotating drum collector at 10-12 kV. Distance betweem needle and collector surface – 15 cm. Feed rate of the polymer solution - 0,6 ml /h.

Biodegradation: samples of all material types were immersed in a phosphate buffer solution (PBS) at pH 7,4 at 37°C with an addition of sodium azide, which prevents microbial growth. After fixed time intervals (1, 3, 7, 30, 90 days), samples were taken from PBS, rinsed thoroughly in demineralized water and then left to dry in a vacuum for a minimum 72 hours.

Analysis: weight loss, scanning electron microscopy (SEM), FTIR, water contact angle, uniaxial tensile testing, DSC.

RESULTS

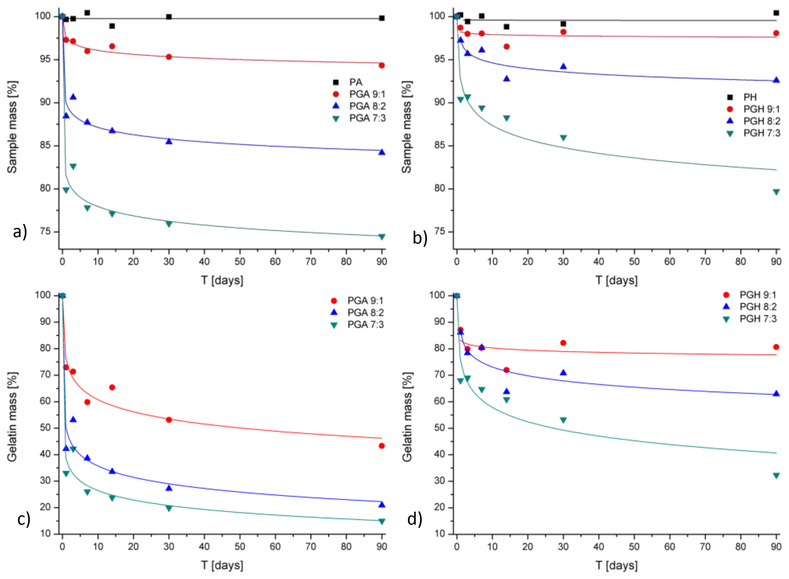


Fig. 2. Overall mass that is left after biodegradation of the samples from a) AA/FA, b) HFIP. The mass of gelatin left after biodegradation in the samples from c) AA/FA, d) HFIP.

Fig. 2 a) and b) shows no mass loss for pure PCL samples. Because of that, it can be assumed that the weight decrease observed for PCL/Gt samples comes from gelatin leaching only. Mass loss is visibly faster for PCL/Gt materials from AA/FA than from HFIP. The greatest amount of gelatin is lost in the first 24 hours and then it slows down.

For both solvents types, the higher the initial gelatin content is, the faster gelatin leaching progresses, as can be seen in Fig. 2 c) and d). After 90 days of biodegradation, for PGA 9:1 there is 43% of gelatin mass left, while for PGA 7:3 it is only 15%. For PGH 9:1 and PGH 7:3 it is 81% and 32% respectively.

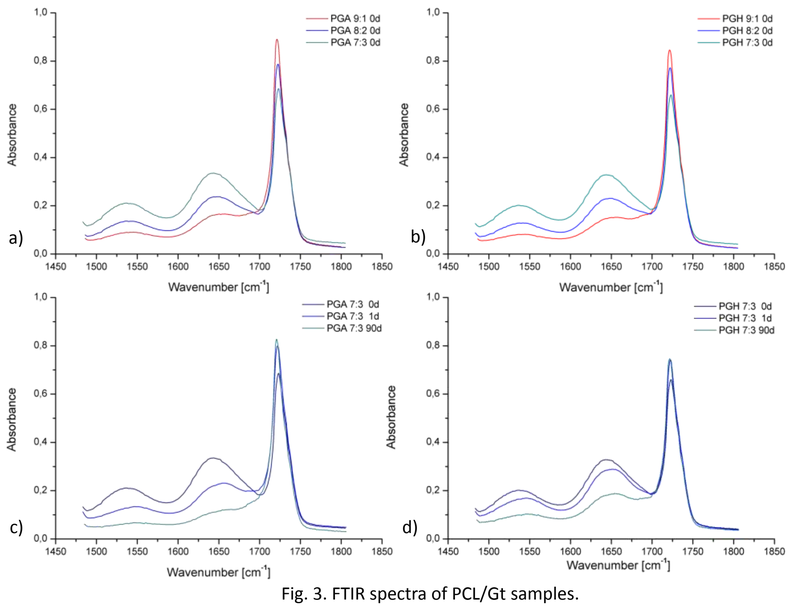


Fig. 3. FTIR spectra of PCL/Gt samples.

Fig. 3. a) and b) shows that for both types of solvents there are no differences in Amide I and II bands at 1650 and 1530 cm^{-1} (characteristic for collagen and collagen derived materials) for the samples with the same gelatin content before degradation.

Fig. 3. c) and d) that characterize only PCL/Gt 7:3 nonwovens proves faster gelatin leaching from AA/FA samples what is evident from more rapidly diminishing areas of Amide I and II in time (0d compared to 1d and 90d). This result interacts with results of mass loss tests.

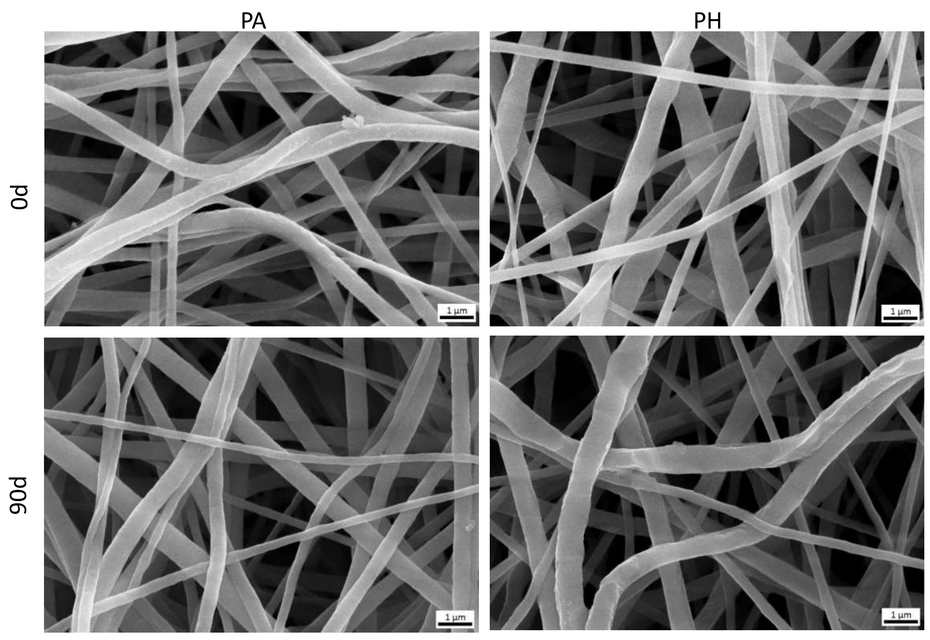
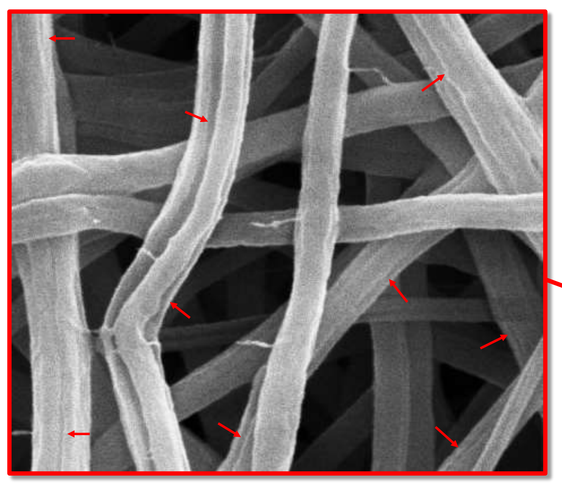


Fig. 4. SEM images of pure PCL nonwovens from AA/FA (PA) and HFIP (PH), before (0d) and after 90 days of biodegradation.



Nonwovens electrospun from both solvents have similar morphology, that for pure PCL samples does not change even after 90 days of biodegradation experiment (Fig. 4).

Fig. 5. shows the differences in surface structure of PGA 7:3 and PGH 7:3 after 1 and 90 days in PBS in 37°C. Red magnified fragment shows a distinct gelatin loss from the fibres surface that appears after just 24 hours of biodegradation. Image reveals linear groove-like sites remaining after gelatin leaching (red arrows).
Fibres spun from HFIP remain smooth even after 90 days of experiment with only 32% of gelatin mass left.

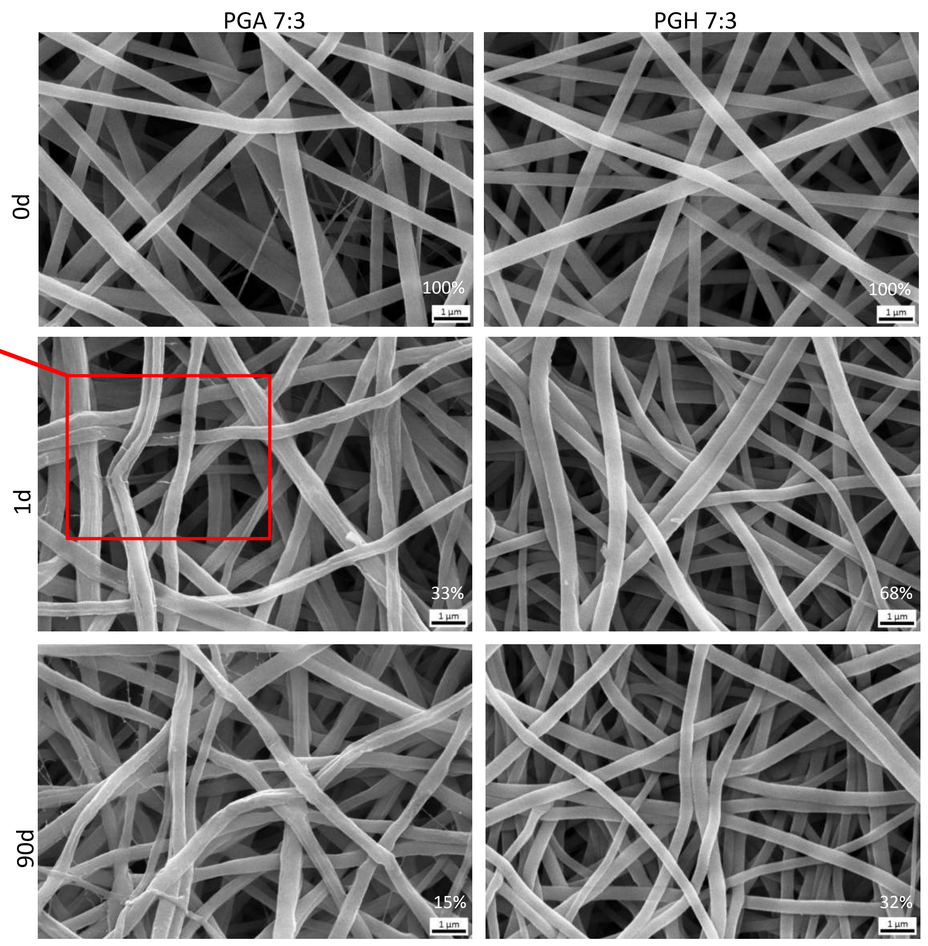


Fig. 5. SEM images of PCL/Gt 7:3 nonwovens from AA/FA (PGA 7:3) and HFIP (PGH 7:3), before (0d) and after 1 and 90 days of biodegradation. Percentages of gelatin mass above scale bars.

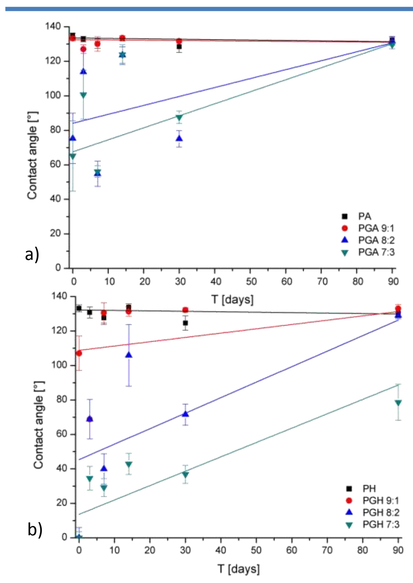


Fig. 6. Water contact angle measurements.

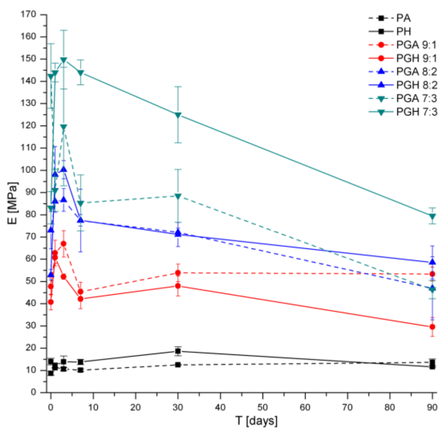


Fig. 7. Young's modulus calculated from uniaxial tensile testing.

Gelatin depletion from the fiber surface results in increasing hydrophobicity in time, what was measured by water contact angle tests (Fig . 6). The nonwovens with higher gelatin content in the beginning of biodegradation experiment exhibit lower contact angles, that increase over time. This trend is more pronounced for PCL/Gt from HFIP. After 90 days of experiment all materials, except for PGH 7:3 have contact angle around 130 degrees.

Fig. 7. shows Young's modulus values, calculated from uniaxial tensile testing results. The more gelatin there is in the material, the higher Young's modulus is, though for the same gelatin content, samples from HFIP exhibit higher values. Due to the fact that from these two components (PCL and Gt) it is gelatin that has higher Young's modulus on its own, as it is leached out over time, there is general trend of reduction of Young's modulus for all samples.
In the beginning of the biodegradation experiment (from day 1 to 3) Young's modulus values of all materials with gelatin exhibited temporary increase. It is most probably caused by gelatin conformation changes that occur in aqueous medium.

CONCLUSIONS

Bicomponent PCL/Gelatin nanofibres electrospun from the mixture of acetic acid and formic acid, as well as from commonly used perfluorinated alcohol HFIP exhibit similar morphology, but their structure and properties differ. It is even more evident for nonwoven samples after biodegradation experiment.

- Faster gelatin loss, higher water contact angles and lower Young's modulus values are observed for materials spun from AA/FA. It can be explained by nonuniform distribution of gelatin within fibres due to emulsive character of solution.
- It is evident from SEM images that, within the fibre, gelatin is in the form of strings that are much more prone to be easily leached out than gelatin being molecularly dispersed in polycaprolactone, how it happens in case of electrospinning from perfluorinated alcohols. Fibres made from HFIP remain smooth even after substantial gelatin depletion for the same reason. Well dispersed gelatin being leached out does not cause changes on fibres surface.
- Mass measurements, FTIR profiles, as well as SEM images indicate very fast gelatin leaching from all material types in the first 24 hours of biodegradation, followed by slower rate stage.
- The smaller initial gelatin content is, the better gelatin is preserved. This, coupled with slower gelatin loss from HFIP materials, leads to conclusion that PGH 9:1 is the most effective material in retaining gelatin, as after 90 days of biodegradation there is still 8% of gelatin , while PGA 7:3 has only 6% (Fig. 8).

Biodegradation experiment simulates in-vivo conditions. Gelatin loss that occurs during this test impairs biological and mechanical properties of bicomponent PCL/Gt nonwovens. The need to stabilize gelatin within the fibre requires a systematic study and defining optimal crosslinking conditions.

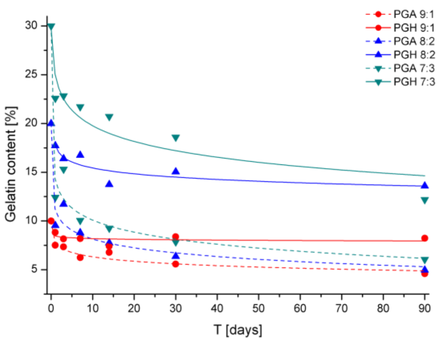


Fig. 8. Comparison of gelatin content over biodegradation time.

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