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Assuring Quality of Scaffolds in Musculoskeletal Tissue Engineering

Mueller Matrix Polarimetry and Transillumination Imaging

Abstract: In order to achieve the high quality required in medical products, reliable characterization methods and quality management systems are necessary. In the field of musculoskeletal Tissue Engineering (mTE), electrospinning is utilized to manufacture fibre scaffolds as implant material. Depending on the application, in this case the regeneration of tendon-bone junctions, properties like the degree of fibre orientation, homogeneity of fibre throughout the scaffold and reaction to external mechanical load are of particular importance. Currently, destructive methods, like scanning electron microscopy (SEM), are widely used to determine these properties. In addition to the destruction of the samples, these methods often only allow the investigation of very small sections. In this study, we present two new methods for the fast, non-destructive and contactless characterization of electrospun fibre scaffolds for mTE. These methods are based on Transillumination Imaging (TI) and Mueller Matrix Polarimetry (MMP), utilizing low-power laser sources or LED light sources, respectively, to determine the relative homogeneity (TI) and the degree of fibre orientation (MMP) in electrospun fibre scaffolds.

Keywords: Tissue engineering, Mueller matrix polarimetry, transillumination imaging, fibre scaffolds, fibre alignment, relative scaffold homogeneity, polycaprolactone, electro-spinning

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1 Introduction

Due to the growing demand for donor organs, modern medicine increasingly relies on Tissue Engineering (TE) [2,3]. Depending on the tissues' functionality, the micro- and macroscopic fibre structure of the extracellular matrix (ECM) distinctly varies. In order to regenerate these tissues artificially, polymers are often used in the field of TE.

The manufacturing of fibre scaffolds via electrospinning technology (see Figure 1) led to promising results so far. The polymers and solvents used (e.g., polycaprolactone (PCL) in trifluoroethanol (TFE) or polyethylene oxide in water) have a huge impact on the resulting structures but also offer an almost unlimited potential for combinations [4,5]. The diameters of the resulting fibres range from nanometres to micrometres [5]. In order to recreate complex tissues such as tendon–bone junctions, fibre scaffolds with varying fibre alignment and diameters are needed [6].

In musculoskeletal TE (mTE) the scaffolds' reaction to external mechanical load is of special interest. This behaviour is strongly influenced by properties such as fibre diameter, degree of fibre orientation and homogeneity of the electrospun fibre scaffold [2]. In order to assess these scaffold properties, and therefore assure its quality, non-destructive and fast examination methods are necessary [1]. In this study, we propose two new methods, based on Mueller Matrix Polarimetry (MMP) and Transillumination Imaging (TI), for the fast and reliable assessment of fibre scaffolds in non-contact mode.

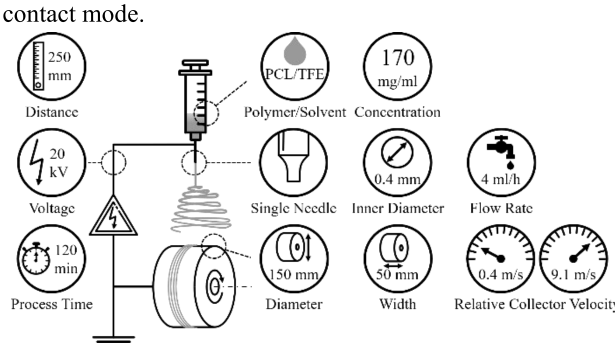

Figure 1: Electrospinning device with process parameters and settings used in this study, adapted from [1]. The samples were fabricated with a process duration of 120 min, 250 mm tip-to-collector distance, a voltage of 20 kV and relative collector velocities of 2 m/s (TI) as well as 0.4 m/s to 9.1 m/s (MMP).

2 Materials and Methods

2.1 Electrospinning

Fibre scaffolds were fabricated using PCL (80 kDa, Sigma-Aldrich Chemistry Corporate, St. Louis, MO, US) in 2,2,2-TFE (99.8%, abcr GmbH, Karlsruhe, Germany) with a concentration of 17% (w/v) (170 mg/mL) (Figure 1) [1]. Due to inhomogeneities in the electrical field distribution, the fibre deposition on the collector could be irregular throughout the fibre scaffold.

2.2 Mueller Matrix Polarimetry

A non-destructive, optical method to generate information about the quality of scaffolds is the measurement of the Mueller Matrix (MM). It contains all information about the polarization changing properties of a sample. If the MM (M_{MM}) of an object is known, it is possible to calculate the Stokes vector of the outgoing light ($\vec{S}_o$) for every incoming light vector ($\vec{S}_i$) by using:

$$\vec{S}_o = M_{MM} \cdot \vec{S}_i \qquad (1)$$

From Eq. (1) one can infer that it is possible to experimentally determine the MM of an object by illuminating the target with polarized light and performing certain intensity measurements in the outgoing path [7]. The measurements can in general be performed by detecting the transmitted or the reflected light from the target.

For electrospun PCL we could show previously, that the distribution of individual fibres within the scaffolds correlates with the MM of the fibre samples [1]. Other experiments have shown, that the results are transferable for samples containing different substances like polycaprolactone:gelatin (PCL:GT) fibre scaffolds [4].

For mTE the orientation of fibres within the scaffold is an important quality characteristic, because the fibre orientation is correlated to physical properties like the tensile strength [1], which is of high importance for the application in tendon-bone junctions.

2.3 Transillumination Imaging

Another non-destructive optical method for the characterization of fibre scaffolds is Transillumination Imaging (TI) [8]. Here, light absorption of a sample is used to determine the relative homogeneity of fibre deposition throughout the fibre mat. The general setup is relatively simple and consists of a light source, camera and computation unit with image analysis software (see Figure 2).

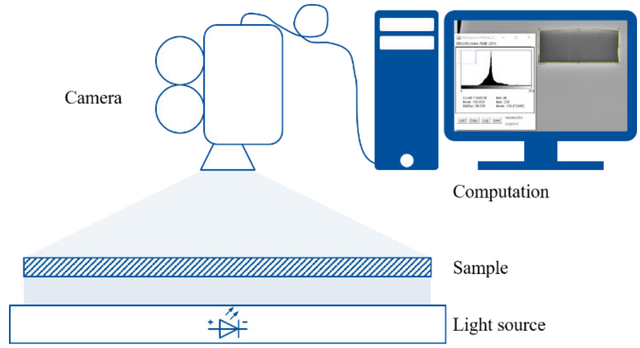

Figure 2: Used TI setup, with LED light source, sample, digital camera and computer with imageJ software.

The fibrous samples (width of 50 mm and length of 150 mm) are placed onto the LED light source (600 mm x 600 mm, 2300 lm, 34 W, floalt, IKEA Deutschland GmbH & Co. KG, München, Germany). An image is taken (I) with the camera (D600, macro 0.25 m efs 18-55 mm, Canon Deutschland GmbH, Krefeld, Germany) (see Figure 3). This

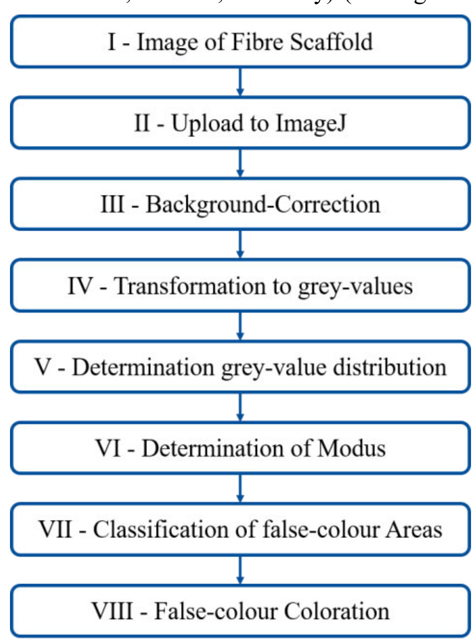

Figure 3: Protocol of the TI approach from imaging (step I) to false-colour coloration (step VIII).

image is loaded into the image analysis software (IAS, imageJ). Initially a background correction is performed for normalization purposes (II, III). The resulting image is transformed into an 8-bit grey-value image (IV). Then the grey-value distribution is determined, utilizing the IAS (V). Based on the modus, the most frequent value, the grey-scale values are evenly classified into intervals (VI). Starting from these intervals, the classification of the false-colour areas is carried out (VII): the first interval (±7% of the modus) is represented in green, the second in light yellow, the third in yellow, the fourth in orange and the fifth one in red. These colours were chosen to enable an intuitive and distinctive determination of the relative homogeneity. For applicants with red-green colour blindness, a second coloration protocol with blue, light blue and yellow was established as well.

3 Results and discussion

3.1 Electrospinning

Fricke & Becker et al. (2019, 2020) already described the relation between the relative collector velocity and the degree of orientation [1,4]. In the wake of these studies, a statistical analysis was carried out. This analysis revealed an increase of the degree of orientation and a decrease of fibre diameter with an increasing relative collector velocity. SEM images of those fibre scaffolds depict the microscopic morphology and dimensions (see Figure 4).

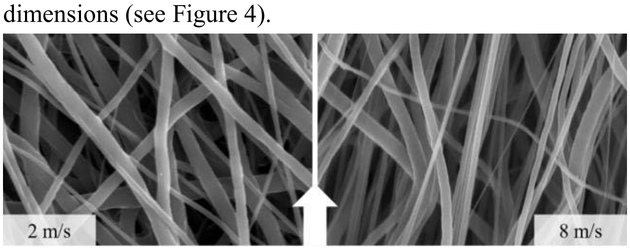

Figure 4: SEM-images of electrospun fibre scaffolds, showing the degree of orientation for different relative collector velocities: 2 m/s on the left side and 8 m/s on the right side. The displayed white arrow represents the rotational direction.

3.2 Mueller Matrix Polarimetry

Fricke & Becker et al.(2019, 2020) [1,4] measured locally resolved MM images of scaffolds manufactured by electrospinning as described above. The same area in the middle of each electrospun sample was measured and homogeneous results in the resulting locally resolved MM images were found. For the final analysis the average MM values of the images were taken and plotted. However, the inhomogeneous areas on the edge of the samples were not included in the analysis. As the MM measurement system presented in [1,4] is already able to measure the locally resolved MM, more systematic measurements on a larger number of samples can be performed in the future.

Most recently Delp & Becker et al. [2] showed the significance of the degree of fibre orientation for mechanical testing of electrospun fibre scaffolds. This supports the findings of Fricke & Becker et al. (see Figure 5) and underlines the importance of MMP for fibre scaffold characterisation.

The area investigated was approximately 10 mm x 13 mm, prepared from the middle region of the fibre scaffolds.

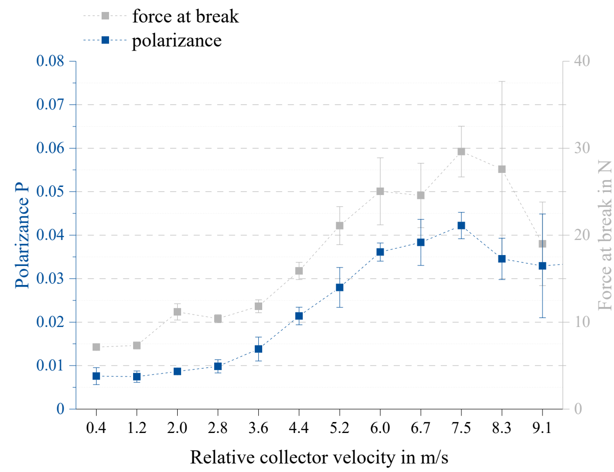

Figure 5: Correlation between force at break and polarizance P measured with MMP. The grey squares (right y-axis) represent the result for force at break and the blue squares depict the results for the polarizance P (left y-axis). The error bars are defined as calculated standard deviations [1].

For comparison, the samples measured with the TI approach have dimensions of 150 mm x 50 mm (see below). Thus, as the TI setup can measure the whole sample including the edges within a single measurement, the utilized MM system would have to scan the sample. In general, the MM method could be adopted to the sample size to realise the measurement of the locally resolved MM of the whole sample within a single measurement by using another camera lens and optical components with other dimensions, e.g. diameter. Due to the normalization procedure applied for data analysis, i.e. all matrix elements are normalized by the first MM element, a dependency of the results on the samples' thickness is not to be expected.

3.3 Transillumination Imaging

The protocol described in Section 2.3 results in a sequence of images (see Figure 6) from each sample, analysing fractions or the whole fibre scaffold (470 mm x 50 mm) to determine the relative distribution of the deposited fibres. With the implemented background-correction, the influence of the LED light source on the results is decreased. Thus, an 8-bit grey-scale value image is representative for the absorption of the fibre scaffold sample itself. Homogeneity of the LED light source was investigated and showed a deviation of less than 3%.

Fricke & Becker et al. (2019, 2020) [1,4] performed MMP measurements on samples prepared from the middle region of electrospun fibre scaffolds. The investigation of the relative homogeneity by TI, presented in this work, confirms the hypothesized reproducibility of the samples investigated.

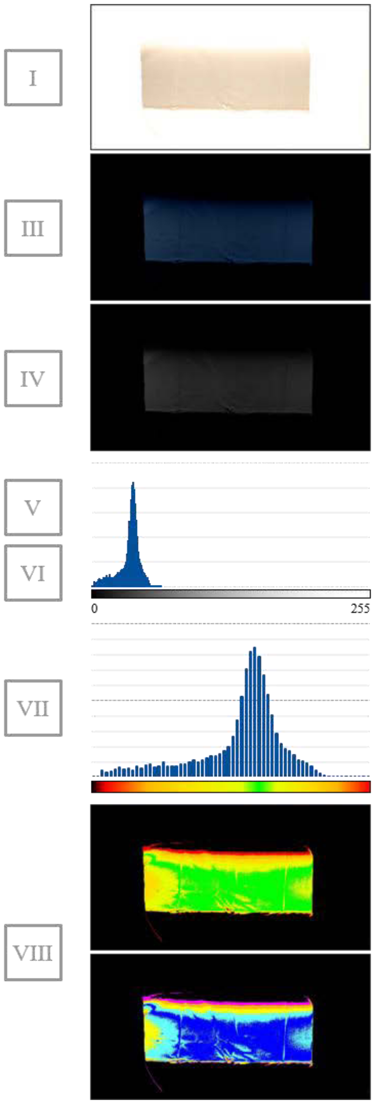

Figure 6: Results of the TI protocol as image sequence: image of the fibre scaffold (I), background-corrected image (III), transformed 8-bit grey-scale value image (IV), determination of grey-scale value distribution and modus (V, VI), classification of false-colour areas in green/yellow/orange/red (VII) and false-colour TI images in green/yellow/orange/red and blue/light blue/yellow (VIII).

A further development step should include a revision of the intervals used for false-colour area classification and an approach on the automation of the software-based analysis, for example with a dedicated algorithm.

4 Conclusion

In this work, we present two methods for the analysis of engineered fibre scaffolds. The methods, MMP and TI, operate with low-power laser sources or LED light sources, respectively. Thus, the energy input in the samples is not significant. In contrast to SEM-based methods, both approaches leave the samples unchanged. Additionally, the characterization of whole fibre scaffolds is possible, with the MMP in scanning mode allowing for higher precision compared to SEM-based analysis. These methods constitute powerful tools for the fast, non-contact and non-destructive characterization of electrospun fibre scaffolds.

Author Statement

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Conflict of interest: Authors state no conflict of interest.

References

[1] Fricke D, Becker A, Jütte L, Bode M, Cassan D de, Wollweber M et al. Mueller Matrix Measurement of Electrospun Fiber Scaffolds for Tissue Engineering. Polymers 2019;11(12).

[2] Delp A, Becker A, Hülsbusch D, Scholz R, Müller M, Glasmacher B et al. In Situ Characterization of Polycaprolactone Fiber Response to Quasi-Static Tensile Loading in Scanning Electron Microscopy. Polymers 2021;13(13):2090.

[3] Hunsberger J, Neubert J, Wertheim JA, Allickson J, Atala A. Bioengineering Priorities on a Path to Ending Organ Shortage. Curr Stem Cell Rep 2016;2(2):118–27.

[4] Fricke D, Becker A, Heratizadeh A, Knigge S, Jütte L, Wollweber M et al. Mueller Matrix Analysis of Collagen and Gelatin Containing Samples Towards More Objective Skin Tissue Diagnostics. Polymers 2020;12(6).

[5] Suresh S, Becker A, Glasmacher B. Impact of Apparatus Orientation and Gravity in Electrospinning-A Review of Empirical Evidence. Polymers 2020;12(11).

[6] Gniesmer S, Brehm R, Hoffmann A, Cassan D de, Menzel H, Hoheisel AL et al. Vascularization and biocompatibility of poly(ε-caprolactone) fiber mats for rotator cuff tear repair. PLOS ONE 2020;15(1):e0227563.

[7] Bass M (ed.). Handbook of optics, 2nd ed. New York, NY: McGraw-Hill; 1995.

[8] Sundermann J, Oehmichen S, Sydow S, Burmeister L, Quaas B, Hänsch R et al. Varying the sustained release of BMP-2 from chitosan nanogel-functionalized polycaprolactone fiber mats by different polycaprolactone surface modifications. Journal of biomedical materials research. Part A 2021;109(5):600–14.