Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: http://ees.elsevier.com

Out-of-plane auxetic nonwoven as a designer meta-biomaterial

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ARTICLE INFO

Keywords
Biomaterials
Nonwoven
Auxetic
Porous materials
Structural
Out-of-plane

ABSTRACT

Biomaterials are porous and three-dimensional (3D) templates, which are used as biological substitutes in tissue engineering. Targeting the optimal design of biomaterials requires a synergy between mechanical, porous, mass transport, and biological properties. To address this challenge, we propose a non-periodic meta-biomaterial in the form of an out-of-plane auxetic nonwoven scaffold that possesses a 3D interconnected highly porous structure with remarkable mechanical properties corresponding to conventional nonwoven material. A design strategy of utilizing larger fiber diameters to enhance the porosity and permeability characteristics successfully devised the nonwoven scaffold with an extraordinary out-of-plane auxetic effect. *In situ* tensile-X-ray microcomputed tomography (microCT) analysis has been carried out to monitor the variation in the morphological characteristics.

1. Introduction

Porous biomaterials are used as templates in tissue engineering that revive the damaged tissues and organs by combining biological knowledge and engineering principles. These templates are three-dimensional (3D) in structure, usually seeded with cells and growth factors (Griffith and Naughton, 2002; O'brien, 2011). *Ideally*, the engineering design of 3D porous biomaterial scaffolds should leverage the cell density, extend the cell proliferation period, and enhance the cell differentiation activity (Li and Yang, 2001). Catering these requirements would demand the 3D scaffolds to possess sufficient mechanical properties for surgical handling. In addition, these scaffolds should be highly porous along with a high level of interconnectivity amongst the pores to warrant cellular infiltration and sufficient diffusion of nutrients to cells. However, it is an open challenge for a highly porous structure to retain good mechanical properties (Karageorgiou and Kaplan, 2005). To address this challenge, the presented work aims to introduce an out-of-plane auxetic 3D nonwoven scaffold that exhibits a highly interconnected porous structure with striking mechanical properties in comparison to conventional nonwoven material.

Auxetic meta-biomaterials exhibit negative Poisson's ratio, and accordingly, these materials tend to expand in one of the transverse directions in response to stretching (Kolken and Zadpoor, 2017). Although the majority of the investigated meta-biomaterials consist of periodic and well-ordered structures (Kolken et al., 2020), the non-periodic materials such as nonwovens have been rarely investigated. Nonwoven materials are a unique class of 3D scaffolds that not only offers high porosity but allows a sufficiently strong and mechanically stable structure (Soares et al., 2017). To develop a library of the nonwoven scaffolds with a wide range of geometrical and mechanical properties, it is essential to exploit the out-of-plane auxetic characteristics that allow material expansion in the thru-thickness direction upon stretching. Recently, auxetic scaffolds with tunable negative Poisson's ratio displayed the unusual cell division, and structural reorganization yielded distinct sets of biophysical signals (Yan et al., 2017; Zhang et al., 2013). Therefore, the central goal of the research work was to develop out-of-plane auxetic polypropylene-based nonwoven scaffolds, followed by their morphological and mechanical analyses. Particularly, the morphological and transport characteristics of out-of-plane auxetic nonwoven have been unfolded by *in situ* monitoring of 3D structure via X-ray microcomputed tomography (microCT) analysis. A comparison has also been made between various properties of corresponding conventional and auxetic nonwoven scaffolds.

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https://doi.org/10.1016/j.jmbbm.2020.104069
Received 21 April 2020; Received in revised form 17 August 2020; Accepted 25 August 2020
Available online xxx

2. Materials and methods

Two needlepunched nonwoven materials were prepared using polypropylene fibers with distinct fiber dimensions. Similar to our earlier works (Rawal et al., 2019, 2017), these nonwoven materials were fabricated by opening the fibers, followed by carding and cross-lapping processes that resulted in anisotropic web structures. The webs were then made to pass through two sets of needle boards in a needlepunching process by setting the punch density and depth of needle penetration as 150 punches/cm^2 and 15 mm, respectively. Here, the samples A and B have been assigned to the scaffolds prepared from coarser and finer fibers, respectively (see Table 1). Further, the tensile properties of nonwoven scaffolds with specimen dimensions of 100 mm × 50 mm were determined on an Instron Testing System 3365 that had a load cell of 5 kN. Here, the specimen grip moved under a constant strain rate of 10 mm/min. In addition, on-line Poisson's ratios were determined by capturing the images using two high-resolution digital cameras placed in the width-wise and thru-thickness directions. Here, the virtual rectangles were drawn at the center of the specimen, and the changes in their dimensions were recorded under a 5% level of strain with respect to the unstrained state. Subsequently, ImageJ® was used for the post-processing of captured images in determining the in-plane and out-of-plane Poisson's ratios. Further, the porosity of nonwoven scaffolds was determined by measuring thickness under defined compressive stresses (2.45, 4.9, 9.8, and 19.6 kPa). Table 1 shows a key set of fiber and nonwoven scaffold characteristics.

The morphological analysis of nonwoven scaffolds was primarily carried out using an X-ray micro-computed tomography technique (Bruker SkyScan 2211). In this technique, the nonwoven specimens of dimensions 25 mm × 5 mm were mounted in the strain cell of the X-ray microCT equipment and were scanned at unstrained and 5% strain level. The X-rays were focused using a tungsten target with a 50 kV source voltage and 600 μA current. The sample stage was rotated up to 180° in 1200 steps of 0.15°each to obtain 2300-2400 projected images after 600 ms of X-ray exposure. The projections obtained were then reconstructed using the commercially available NRecon (SkyScan, Bruker, Belgium) software. The artefacts in the projections were removed using beam hardening correction, defect pixel masking, and ring artefact reduction techniques. 3D volumes of the specimens were created using CTVOX® (Bruker, Belgium) and AVIZO® (evaluation version). The reconstructed images were analyzed for fiber orientation distribution using CTAN® software (Shukla et al., 2020). Pore interconnectivity, pore volume distribution, air permeability of nonwoven scaffolds in the thru-thickness direction were determined using Avizo® (evaluation version). Specifically, the air permeability of nonwoven scaffolds was determined under a pressure drop of 100 Pa.

Table 1
Nonwoven scaffolds and their fiber characteristics.

Properties	Sample A	Sample B
Fiber diameter (μm)	48	30
Fiber length (mm)	60	51
Nominal mass/area (gm $^{-2}$)	600	600
Thickness (mm)	5.30	7.6
Elastic modulus (MPa)	3.03 ± 0.37	1.44 ± 0.13
Specific modulus (MPa kg $^{-1}$m ^{3})	0.03 ± 0.003	0.02 ± 0.002
Tensile strength (MPa)	5.00 ± 0.23	3.80 ± 0.63
Breaking strain (%)	147.15 ± 14.31	84.87 ± 11.72
Toughness (MPa)	4.45 ± 0.46	1.35 ± 0.35
In-plane Poisson's ratio	2.13 ± 0.13	0.99 ± 0.28
Out-of-plane Poisson's ratio	−3.99 ± 0.23	1.52 ± 0.70
Initial porosity	0.88	0.91
Porosity at 5% strain	0.90	0.91
Change in Volume (%)	12.52	−4.39

3. Results and discussion

In the past, the importance of 3D microporous scaffolds has been highlighted to promote 3D cell-cell interactions that effectively modulated the cell-specific function (Li and Yang, 2001). With the aid of X-ray microCT analysis, we unravel the 3D structure of a needlepunched nonwoven scaffold, as illustrated in Fig. 1a and b. In a nonwoven scaffold, some of the fibers are aligned in the thru-thickness direction that can potentially allow the 3D cellular migration and reorganization (Li and Yang, 2001). Though, the majority of the fibers are aligned in the in-plane direction, as depicted in Fig. 1c and d, and Fig. S2. Intriguingly, the sample A prepared from larger fiber diameters displayed large negative Poisson's ratio in the thru-thickness direction, as shown in Table 1. Here, the larger fibers present in the thru-thickness direction can generate a significant level of transverse forces on the in-plane fibers leading to the 'bulging' effect (Rawal et al., 2019, 2017). Given the fact that there is a marginal increase in the porosity, the tensile properties of sample A are still significantly higher than that of a conventional scaffold produced under similar process conditions (see Table 1 and Fig. S1). However, sample A maintained its structural integrity similar to sample B under compression loading. Since the reduction in the porosity of sample An under compression loading is similar to that of sample B, as shown in Fig. S3.

Porosity is an established determinant for creating any scaffold successful for tissue engineering applications (Soliman et al., 2011). Fig. 2a demonstrates greater coverage of pore volume in an out-of-plane auxetic nonwoven scaffold. Under the unstrained state, the porosity of sample B appears to be higher than that of an auxetic nonwoven scaffold (see Table 1). Though, the porosity value of sample A approaches to that of a conventional scaffold at a 5% level of tensile strain. Upon stretching, there is an overall increase in the volume of sample A whereas there is a reduction in the volume of sample B. Sample A has an added advantage that they are made up of fibers with significantly larger diameters (~60%) than the fibers used in sample B. According to Takahashi and Tabata (2004), relatively more mesenchymal stem cells were found to be attached to the nonwoven fibers with larger diameters than the thinner ones. Another demand of the state-of-art in scaffolds is to choose a uniform nonwoven in order to avoid fewer cells attached in the regions that are loose or circumvent the formation of cell aggregates in pockets with densely packed fibers (Li and Yang, 2001). To address this challenge, we analyzed the variation in local porosity of nonwoven scaffolds, as shown in Fig. 2b and Fig. S4. Sample A maintains a higher magnitude of porosity along the thickness direction during tensile loading. Such views have also been substantiated based upon the distribution of pore volumes in sample A, as illustrated in Fig. 2c. In sample B, there is a significant reduction in smaller pores ($<$100 μm) followed by a marginal increase in larger pores ($>$400 μm) upon stretching. However, the pore space is uniformly maintained in an auxetic nonwoven scaffold even on application of tensile strain.

The uniformity of pore space should ensure that these pores are highly interconnected not only to allow cellular penetration and adequate diffusion of nutrients to cells but a way forward to extract the waste products from scaffolds (O'brien, 2011). Accordingly, the interconnectivity of pores has been analyzed, and generally, the nonwoven scaffolds displayed a highly interconnected porous structure (see Fig. 2d). The permeability of scaffolds also plays a key role in the nutrient and waste transportation within the structure (O'Brien et al., 2007). This has directed us to compute the thru-plane permeability characteristics of nonwoven scaffolds, as shown in Fig. 3.

High magnitude of porosity is expected to yield better permeability characteristics; accordingly, sample B showed higher thru-plane permeability than sample A. On stretching, there is a dramatic change both in

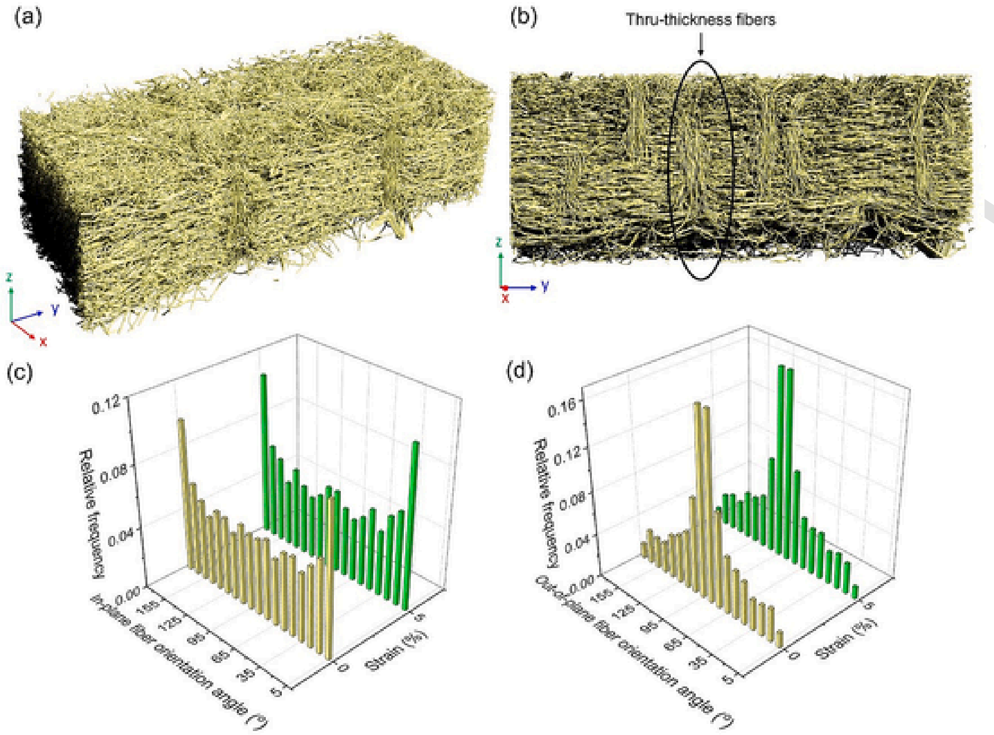

Fig. 1. X-ray microCT analysis of sample A (a) 3D rendered image (b) Cross-sectional view in the YZ plane (c) In-plane fiber orientation distribution (d) Out-of-plane fiber orientation distribution. Here, 90° represents machine and planar directions in the in-plane and out-of-plane fiber orientation distributions, respectively.

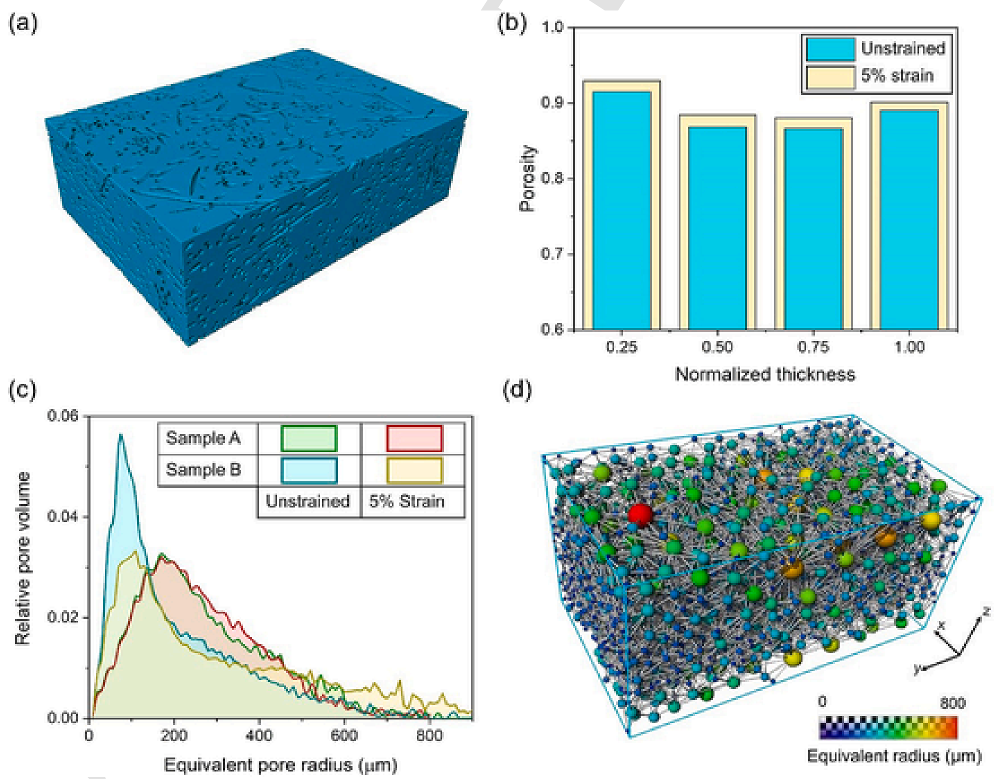

Fig. 2. Pore morphology of nonwoven scaffolds (a) Pore volume (indicated by blue color) (b) Variation of porosity along with the thickness (c) Pore volume distribution of samples A and B under unstrained and strained states, and (d) Pore network model of sample A represented by spheres (end or connection point with another pore) and segments (pore body). The pore volume distributions and pore network models have been generated via Avizo® (Evaluation version). Here, normalized thickness refers to the proportion of the total thickness.

the trend and the magnitude of thru-plane permeability of auxetic nonwoven scaffold. Interestingly, the magnitude of the thru-plane permeability of sample A approaches to that of sample B at a 5% level of strain. Besides the improvement in the thru-plane permeability of out-of-plane auxetic nonwovens, these scaffolds possess significantly higher magnitudes of mechanical properties while preserving the highly

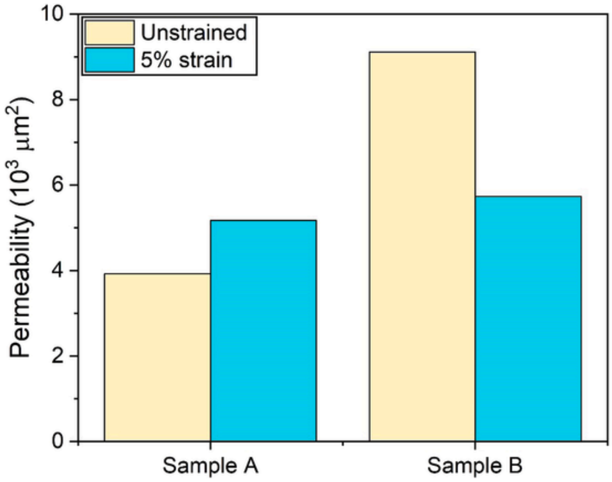

Fig. 3. Thru-plane permeability of samples A and B under unstrained and strained states.

porous architecture and interconnected pore network. In the past, the nonwoven scaffolds have established as excellent templates for tissue-engineered bone or cartilages, but the key challenge is to attain similar mechanical properties as that of host bone or cartilage (Hutmacher, 2000). The interplay between the mechanical properties and porous characteristics of out-of-plane auxetic nonwoven is a step towards achieving these goals.

4. Conclusions

In this research work, the polypropylene-based out-of-plane auxetic nonwoven scaffold was successfully developed using a larger fiber diameter. The insights about the morphological and transport characteristics of nonwoven scaffolds were obtained via X-ray microCT analysis. Subsequently, the merits of out-of-plane auxetic behavior were manifested in terms of enhancement in porosity, mechanical properties, and thru-plane permeability characteristics corresponding to the conventional nonwoven scaffolds. In an out-of-plane auxetic nonwoven scaffold, the pores are not only highly interconnected but maintain pore volume distribution and uniform porosity upon stretching. The versatility provided by these scaffolds can endeavor into bone and cartilage regeneration applications.

Credit authorship contribution statement

Amit Rawal: Conceptualization, Methodology, Writing - Review & Editing, Funding acquisition. **Sumit Sharma:** Methodology, Software, Validation, Visualization. **Danvendra Singh:** Methodology, Software, Validation. **Nitesh Kumar Jangir:** Investigation, Formal analysis. **Harshvardhan Saraswat:** Methodology, Resources. **Dániel Sebők:** Investigation. **Akos Kukovecz:** Conceptualization, Funding acquisition. **Dietmar Hietel:** Conceptualization, Writing - Review & Editing. **Martin Dauner:** Conceptualization, Writing - Review & Editing. **Levent Onal:** Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors gratefully acknowledge the financial support by Indo-Hungarian Joint Research project no. INT/HUN/P-18/2017 (2017–2.3.7-TÉT-IN-2017-00008). The NKFIH GINOP-2.3.3-15-2016-00010 project and a HAS Janos Bolyai fellowship (D.S.) are acknowledged.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jmbbm.2020.104069.

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