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Mechanical and biological properties of electrospun biomimetic dura mater substitute

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1. Introduction

Dura mater is the most external layer of the meninges, a half-rigid membrane crucial for protecting the brain and spinal cord. Because of its role, biomechanical properties are important and should be considered when developing dura substitutes. When dura mater is injured or removed, artificial substitutes are commonly used but most of them lack the complex biological and structural factors of the dura mater's structure which leads to complications, such as cerebrospinal fluid leakage (Bi et al., 2020). Developing a tissue-engineered biomimetic dural model could lead to a better understanding of this tissue, and become both an *in vitro* alternative to animal testing and an innovative implantable substitute as artificial cranial dura. Based on the morphological similarities between the native dural extracellular matrix and polymer fibres, our goal is to develop a multiphasic model mimicking the native dura, from the bone side to the deepest dural meningeal layers, thanks to electrospun scaffolds cultured with stem cells and/or dural fibroblasts.We present here the mechanical and morphological properties of each scaffold and their impacts on biological activities.

2. Methods

2.1 Fabrication of electrospun scaffolds

Three different electrospun solutions were prepared at 12% w/v: polycaprolactone pure (PCL), 90% PCL/10% nanohydroxyapatite (PCL/HAp), and 80% PCL/20% silk fibroin (PCL/SF). Membranes were prepared by electrospinning. Random (Rd) and aligned (Al) fibres were fabricated by changing the collector speed from 300 to 2000 rpm, respectively.

2.2 Morphological and mechanical characterizations

Fibre diameters were determined by SEM. Tensile mechanical properties of scaffolds were evaluated in two conditions: dry and soaked in PBS for 24 h. Young's modulus, ultimate tensile strength, and elongation at break were calculated from the obtained tensile stress-strain curve.

2.3 In vitro analysis

Primary human dural fibroblasts (HDuFs) and immortalized adipose-derived stem cells (ASC52telo) were monocultured in their respective media for 7 days on 3 scaffolds (random and aligned): PCL, PCL/SF and PCL/HAp. Metabolic activity (Alamar Blue) was measured at days 1, 4 and 7, and cell viability (MTS, Live/Dead) at day 7. ALP staining was performed on day 7 in the ASC52telo.

3. Results and discussion

3.1 Electrospun membranes

PCL, PCL/HAp and PCL/SF scaffolds presented a fibre diameter of 477 nm, 403 nm, and 398 nm, respectively. Porosity ranged from 80 to 82%. The aligned fibres showed high alignment with 6.3° of dispersion. Table 1 depicts the elastic modulus of each group.

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Table 1. Elastic modulus of PCL, PCL/HAp and PCL/SF

Emod (MPa)		Dry condition	Wet condition
PCL	Rd	14.89 ± 1.10	12.95 ± 2.27
	Al	19.12 ± 1.90	21.80 ± 2.10
PCL/HAp	Rd	7.53 ± 2.08	4.79 ± 0.45
	Al	11.87 ± 1.94	8.82 ± 0.60
PCL/SF	Rd	20.62 ± 2.05	7.53 ± 0.61
	Al	40.73 ± 6.50	17.04 ± 1.32

E_{mod}, elastic modulus.

According to a literature review conducted by Pearcy et al. (2022), native cranial dura mater presents an elastic modulus of 68.1 MPa. Although PCL-based scaffolds showed lower E_{mod} than native dura, the alignment of fibres and the SF addition led to higher elastic moduli. Assembling all scaffolds as a multilayer substitute may result in improved mechanical properties that better mimic actual tissue.

3.2 In vitro metabolic and viability tests

HDuFs were selected because they are the main cells in the dura mater, whereas ASC52telo are a promising source for tissue engineering that can differentiate into bone cells. Over the week, metabolic activity increased, indicating growth of both cell types on the scaffolds, regardless of their composition (Figures 1A, 1B). Viability tests on day 7 confirmed viability, demonstrating the absence of cytotoxicity (Figures 1C, 1D). Both cell types aligned along scaffold fibres, indicating the influence of scaffold structure on their behaviour. ASC52telo reacted differently to PCL/SF and PCL/HAp, prompting further investigation into potential differences in proliferation or metabolic changes leading to more cell differentiation. Furthermore, ALP revealed early signs of osteogenic differentiation in ASC52telo cells for all scaffolds (Figure 1E). PCL/HAp Rd showed ASC52telo growth to other conditions and promoted early differentiation suggesting its potential for tissue integration on the bone side of the dura. HDuFs exhibited lower metabolic activity at day 7 on PCL/SF Al, compared to PCL Al which appeared to be the most favourable for proliferation. This could suggest a potential change in metabolism and functionality between these conditions. PCL/SF, particularly of interest for the meningeal layer, demonstrated relatively good viability for HDuFs. No controls without scaffolds were conducted since the different percentages of adhesion and available surface at the cell scale differed between 2D and 3D cultures. Additionally, we rather compared the scaffolds with each other to identify the most promising ones, and the condition PCL was used as a control.

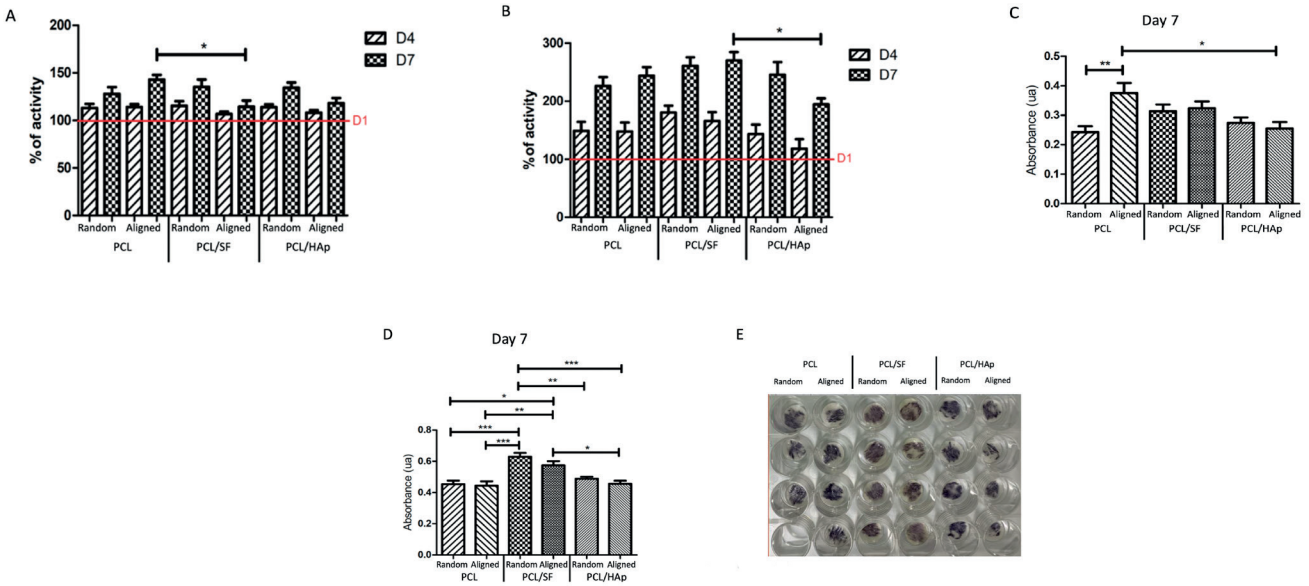

Figure 1. Metabolic activity and proliferative capacity (Alamar Blue assay) of (A) HDuFs and (B) ASC52telo on different scaffolds normalized at day 1 (D1); Viability (MTS assay) of (C) HDuFs and (D) ASC52telo at day 7 on different types of scaffolds. (E) ALP staining of ASC52telo at day 7.

***p<0.001; **p<0.01; *p<0.05 (ANOVA Parametric, post-hoc Tuckey test). Mean and SEM shown.

4. Conclusions

Scaffold designs for each layer of the biomaterial yielded positive outcomes. Their high porosity allowed adequate nutrient diffusion and space for metabolic cell activity. PCL/HAp Rd scaffolds effectively supported ASC52telo growth, viability, and prompted early osteogenic differentiation. Similarly, PCL/SF Al scaffolds supported HDuFs proliferation and viability. The alignment of cells and the presence of SF or HAp appeared to induce functional changes warranting further investigation. These findings shed light on how scaffold composition and alignment impact cell behaviour and fate. Long-term culture will be essential to assess functionality and phenotypic stability, as well as select the best scaffold for each cell type. This approach aims to develop a dura mater model for drug screening and personalized treatments.

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Conflict of Interest Statement

There are no conflicts to declare.

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