**Air-jet spun tissue engineering scaffolds incorporated with diamond nanosheets with improved mechanical strength and biocompatibility**

Robin Augustine1,2\*, Sumama Nuthana Kalva1,2, Yogesh B Dalvi3, Ruby Varghese4, Maneesh Chandran5, Anwarul Hasan1,2\*

1Department of Mechanical and Industrial Engineering, College of Engineering, Qatar University, Doha, Qatar

2Biomedical Research Center (BRC), Qatar University, Doha, Qatar

3Pushpagiri Research Centre, Pushpagiri Institute of Medical Science & Research, Tiruvalla, Kerala-689101, India.

4Department of Chemistry, School of Sciences, Jain Deemed to be University Bangalore, Karnataka 560069, India.

5Department of Physics, National Institute of Technology Calicut, Kozhikode, Kerala 560069, India.

Email: robin@robinlab.in, ahasan@qu.edu.qa

**Histological analysis**

To evaluate the *in vivo* biocompatibility and degradation of PCL scaffolds, with (various concentrations) and without (Blank) DNS in the rat model. PCL scaffolds, with and without DNS scaffold were implanted subcutaneously in the backs of 16 rats (Details are provided in the experimental section of main article). The scaffolds were analyzed histologically at 1st, 2nd, 3rd, and 4th weeks. Sections were viewed and scored by a blinded pathologist using a 4-point scoring system (0 absent, + mild, ++ moderate, and +++ severe) to determine the tissue reaction and scaffold degradation. There was no significant difference in histological scores among PCL scaffolds, with various concentrations of DNS at different time points but high inflammation response and low fibroblast and angiogenesis scores were observed in PCL alone.

**Experimental design**

Before implantation, the PCL scaffolds were cut into 1×1 cm and sterilized (See the experimental section of main article). On the day of surgery, rats were anesthetized by intramuscular injection of 5% Xylazine (10mg/kg of the body weight) and 10% ketamine hydrochloride (80 mg/kg of the body weight). Hairs at the dorsum of the rats were shaved with Philips electric pet care trimmer and skin was cleaned using 2% povidone-iodine solution. A linear incision of 8mm length was made on the dorsum of the rat using carbon steel surgical blade no 15 and the full-thickness flap was elevated. All six sterile scaffolds were implanted and fixed using absorbable 3-0 catgut suture and were closed by giving interrupted sutures with number 3-0 black braided silk. In post-operative care, for three days, ceftriaxone (20 mg/kg) and meloxicam (0.5 mg/kg) were administered IM as an antibiotic and analgesic respectively. The implanted scaffold samples were harvested by euthanizing 4 rats from each group in a CO2 chamber at the 1st, 2nd, 3rd, and 4th weeks. The connective tissue surrounding scaffolds was fixed in 10% formalin for histological evaluation. All sections were stained with Harris’s hematoxylin and eosin (H&E) stain for histological analysis. After H&E staining, sections were investigated histologically by observing under a polarized optical microscope (Leica DM 4500 P LED) and the images were captured. Sections were viewed and scored by a blinded pathologist using a 4-point scoring system (0 absent, + mild, ++ moderate, and +++ severe) to determine the tissue reaction and scaffold degradation.

**Result**

**Table S1:** Cell response scaffolds after 1st weeks of subcutaneous implantation in rats (scored by a blinded pathologist using a 4-point scoring system (- absent, + mild, ++ moderate, and +++ severe).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sr. No.** | **Observations** | **PCL** | **PCL-DNS-0.5** | **PCL-DNS-1** | **PCL-DNS-2** | **PCL-DNS-4** |
| 1 | Fibroblasts | + | + | + | +\_ | + |
| 2 | Angiogenesis | - | + | + | + | + |
| 3 | Necrosis | - | - | - | - | - |
| 4 | Lymphocytes | +++ | +++ | +++ | +++ | ++ |
| 5 | PMNs | ++ | +++ | +++ | +++ | +++ |
| 6 | Giant cells | +++ | ++ | +++ | ++ | ++ |
| 7 | Plasma cells | +++ | ++ | +++ | +++ | ++ |
| 8 | Overall Tissue inflammation response | Moderate | Moderate | Moderate | Moderate | Moderate |

**Table S2:** Cell response scaffolds after 2nd weeks of subcutaneous implantation in rats (scored by a blinded pathologist using a 4-point scoring system (- absent, + mild, ++ moderate, and +++ severe).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sr. No.** | **Observations** | **PCL** | **PCL-DNS-0.5** | **PCL-DNS-1** | **PCL-DNS-2** | **PCL-DNS-4** |
| 1 | Fibroblasts | + | ++ | ++ | ++ | ++ |
| 2 | Angiogenesis | + | ++ | ++ | ++ | ++ |
| 3 | Necrosis | - | - | - | - | - |
| 4 | Lymphocytes | ++ | ++ | ++ | + | ++ |
| 5 | PMNs | +++ | ++ | ++ | ++ | ++ |
| 6 | Giant cells | ++ | ++ | ++ | ++ | ++ |
| 7 | Plasma cells | ++ | + | + | + | + |
| 8 | Overall Tissue inflammation response | Low-Moderate | Low | Low | Low | Moderate |

**Table S3:** Cell response scaffolds after 3rd weeks of subcutaneous implantation in rats (scored by a blinded pathologist using a 4-point scoring system (- absent, + mild, ++ moderate, and +++ severe).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sr. No.** | **Observations** | **PCL** | **PCL-DNS-0.5** | **PCL-DNS-1** | **PCL-DNS-2** | **PCL-DNS-4** |
| 1 | Fibroblasts | + | +++ | ++++ | +++ | +++ |
| 2 | Angiogenesis | + | +++ | +++ | +++ | +++ |
| 3 | Necrosis | - | - | - | - | - |
| 4 | Lymphocytes | ++ | - | + | - | - |
| 5 | PMNs | ++ | - | - | + | - |
| 6 | Giant cells | + | - | - | - | + |
| 7 | Plasma cells | + | - | - | - | - |
| 8 | Overall Tissue inflammation response | Moderate | Low | Low | Low | Low |

**Table S4:** Cell response scaffolds after 4th weeks of subcutaneous implantation in rats (scored by a blinded pathologist using a 4-point scoring system (- absent, + mild, ++ moderate, and +++ severe).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sr. No.** | **Observations** | **PCL** | **PCL-DNS-0.5** | **PCL-DNS-1** | **PCL-DNS-2** | **PCL-DNS-4** |
| 1 | Fibroblasts | + | +++ | ++++ | +++ | +++ |
| 2 | Angiogenesis | ++ | ++++ | +++ | +++ | ++++ |
| 3 | Necrosis | - | - | - | - | - |
| 4 | Lymphocytes | ++ | - | + | - | - |
| 5 | PMNs | + | - | - | + | + |
| 6 | Giant cells | + | - | - | + | + |
| 7 | Plasma cells | + | - | - | - | - |
| 8 | Overall Tissue inflammation response | Low | Low | Low | Low-Moderate | Low-Moderate |