Supplementary Information

Evaluating the Efficiency of Touch-spun Scaffolds in Producing Dense Cell Cultures for Tissue Engineering Applications

Kristina Peranidze [a,b], Mikhail Parker [a,b], Mohammad Aghajohari [a,b], Polina Vertegel [a,b], Sergei Makaev [a,b], Nataraja Yadavalli [c], Sergiy Minko [a,b,c,*] and Vladimir Reukov [a,*]

[a] Textiles, Merchandising and Interiors, University of Georgia, Athens, GA 30605, USA
[b] Nanostructured Materials Laboratory, University of Georgia, Athens, GA 30602, USA
[c] CytoNest, Inc., 425 River Road, Athens, GA 30602, USA
[*] Correspondence: sminko@uga.edu, Reukov@uga.edu

Materials

In this study, three groups of scaffolds were prepared from 5 wt % PCL/0.25 wt % PEO solutions in chloroform. Using static light scattering (BI-APDX; Brookhaven Instruments, USA), hydrodynamic radii (R_h), gyration radii (R_g), and polydispersity index (PDI) were assessed for commercial PCL ($M_n = 80 \times 10^3$ Da) and PEO ($M_v = 5 \times 10^6$ Da):

	R_h	R_g	PDI
0.5 wt % PCL in chloroform	8.4	11.2	0.418
0.5 wt % PEO in chloroform	41.5	35.0	0.385

The viscosity of the working PCL/PEO solution was evaluated using a modular compact rheometer (MCR 302; Anton Paar, USA). Measurements were performed in two modes: (i) frequency sweep tests at strain amplitudes of 20% and 40%, and (ii) amplitude sweep tests at angular frequencies of 1 and 10 rad/s. Experiments were conducted using 30 mL of solution and a measuring plate (PP25/S) with a plate diameter of 25 mm.

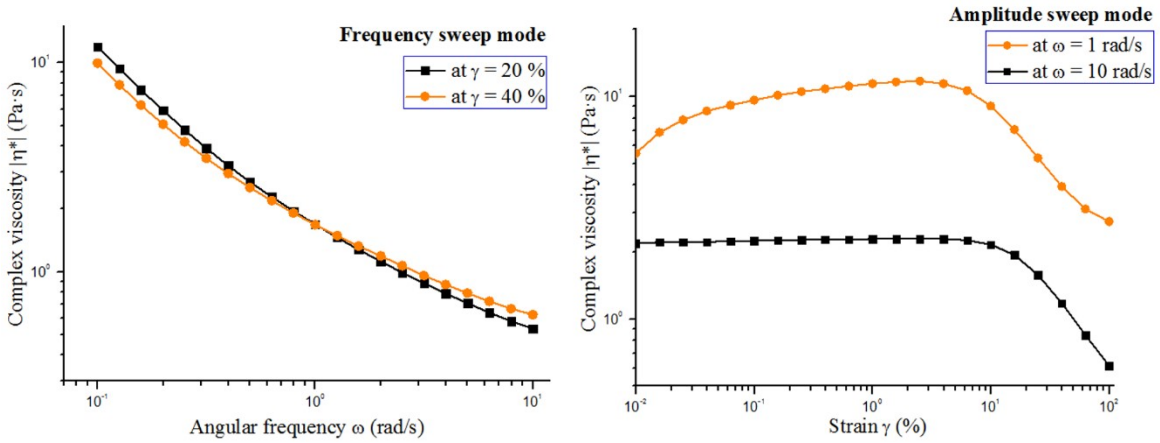


Figure S1. Viscosity measurements obtained in the frequency sweep (left) and amplitude sweep (right) modes. Both plots are presented on a logarithmic scale.

The viscosity data clearly demonstrate shear-thinning behavior, characteristic of non-Newtonian fluids, where the polymer network progressively disentangles under applied shear stress, reducing internal friction. As a result, the viscosity decreases with increasing shear rate. As shown in the frequency sweep plot (left), the complex viscosity at a low angular frequency of 0.1 rad/s ranges from 9.9 to 11.9 Pa·s, whereas at a higher angular frequency of 10 rad/s it drops markedly to 0.5-0.6 Pa·s. A zero-shear viscosity plateau can be identified in the amplitude sweep plot obtained at 10 rad/s (right), with an approximate viscosity value of 2.3 Pa·s.

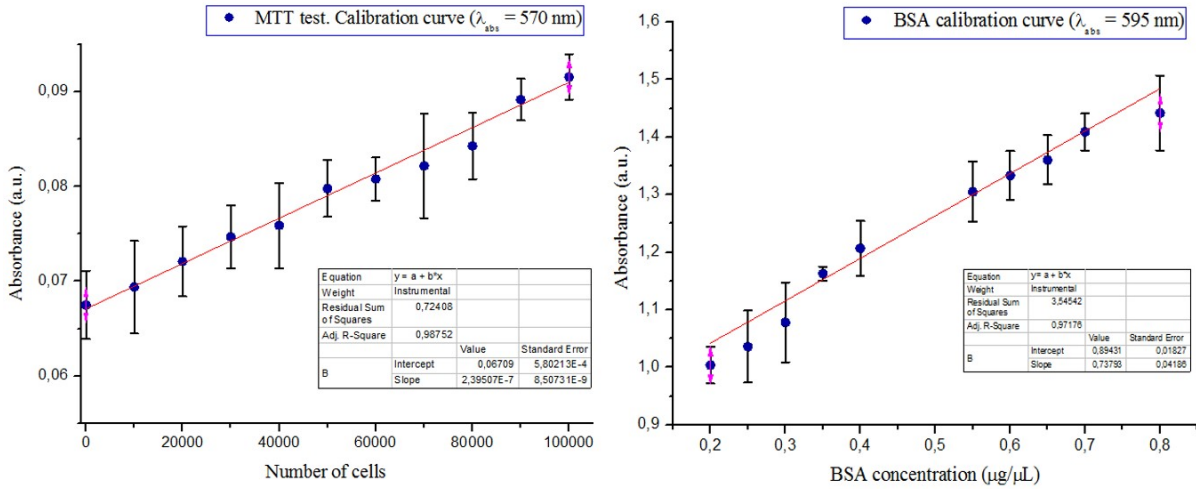


Figure S2. MTT (left) and Bradford (right) calibration curves

Cell numbers estimated from the MTT test for the scaffolds after 10 days of culture were ~ 2.382 × 10^6 cells per sample ('flat surfaces'), ~ 2.597 × 10^6 cells per sample (single-layer touch-spun scaffolds), and ~ 3.195 × 10^6 cells per sample (3D touch-spun scaffolds).

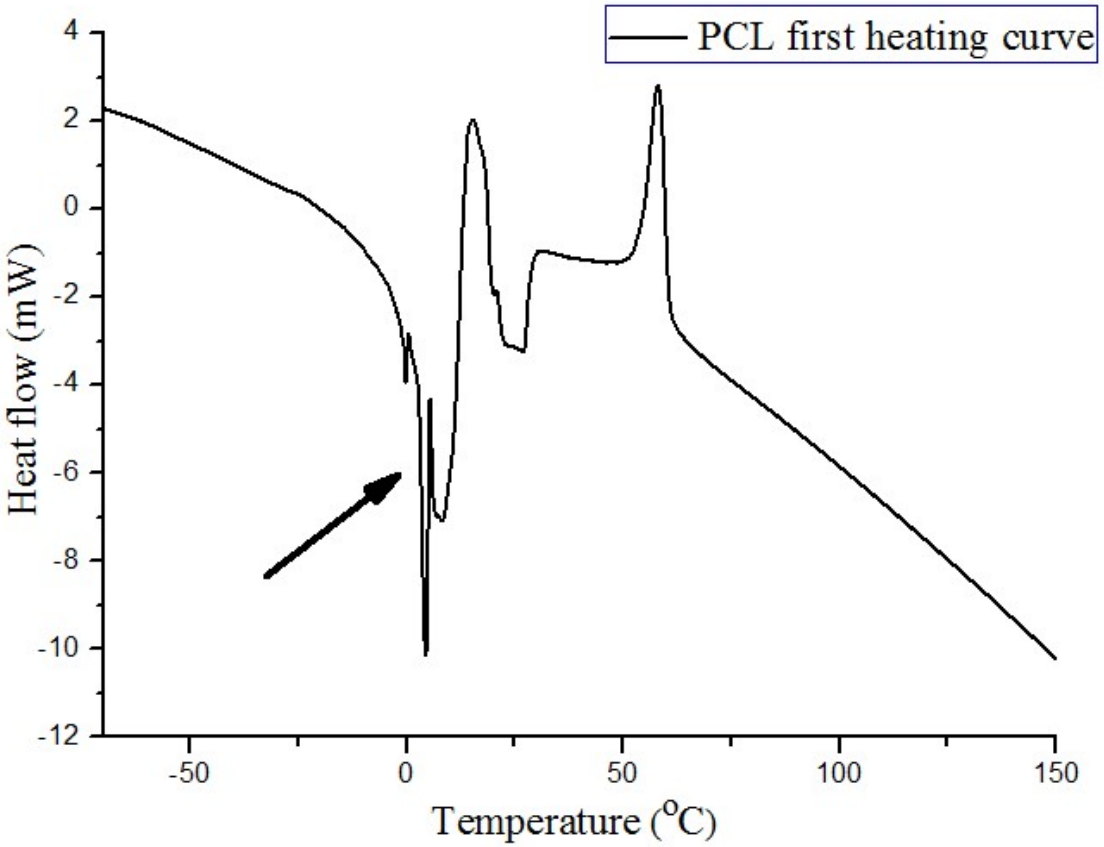


Figure S3. DSC first heating curve obtained for reference PCL nanofibers

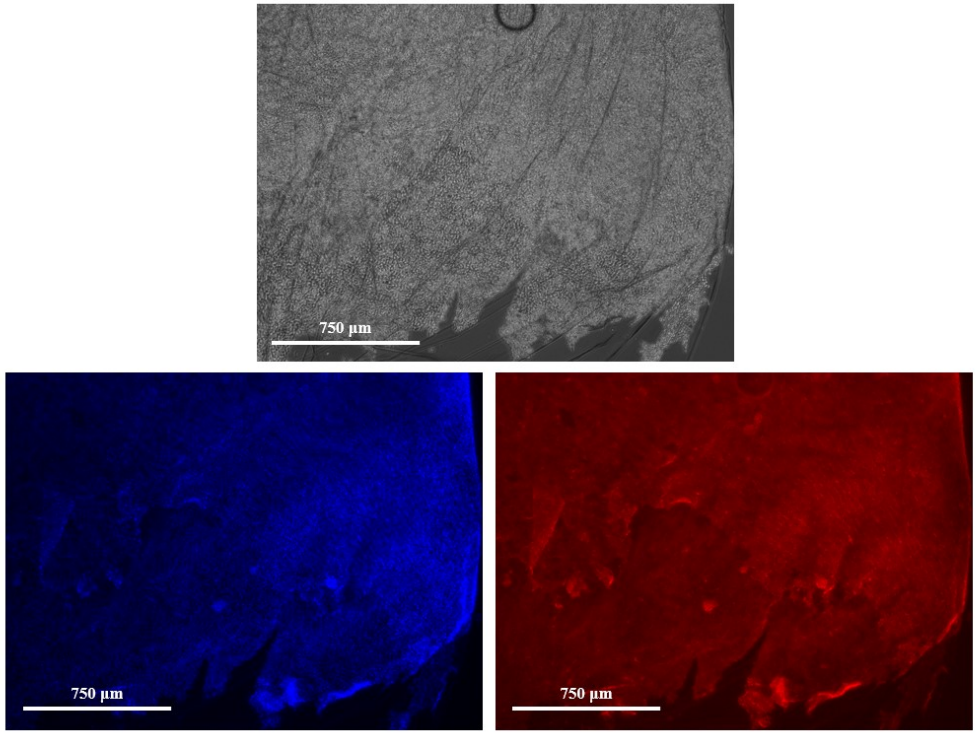


Figure S4. Fluorescence microscopy images of a detaching 3T3-GFP cell sheet after staining (TRANS, DAPI, and RFP channels)

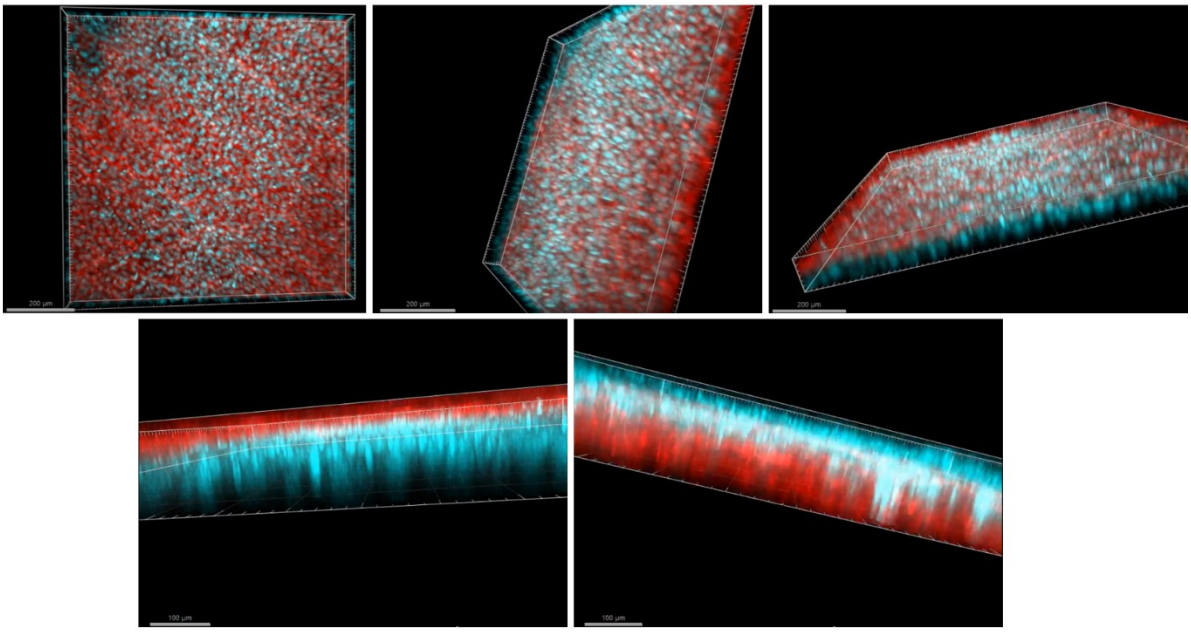


Figure S5. Confocal microscopy images of a 3D scaffold after staining for nuclei and actin. Five representative 3D images were obtained by Z-stack imaging in DAPI and RFP channels

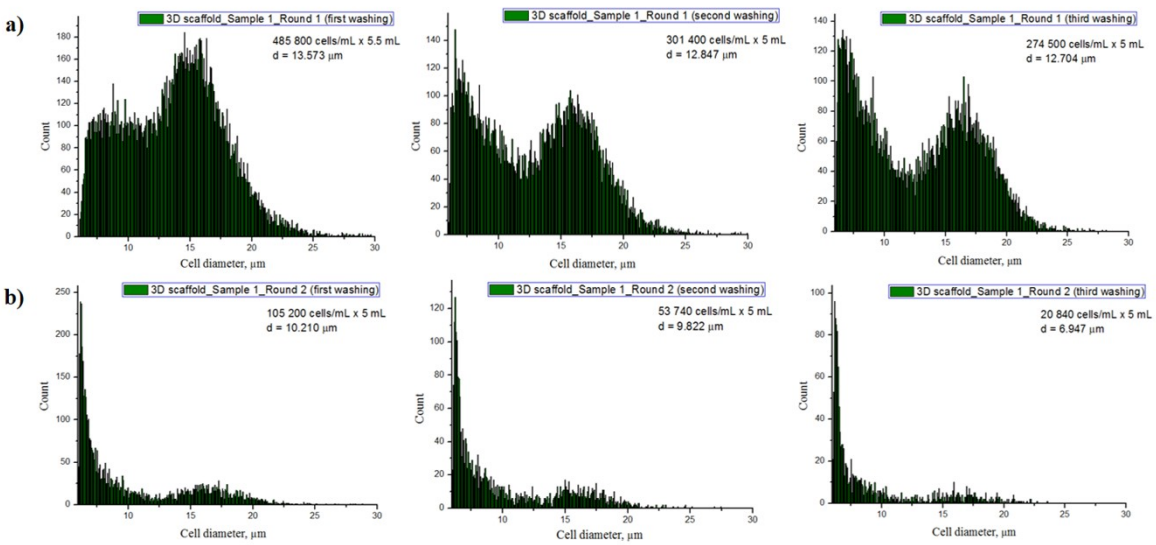


Figure S6. Cell diameter distribution histograms obtained for a 3D scaffold: (a) first and (b) second trypsinization rounds, each followed by three PBS washing steps

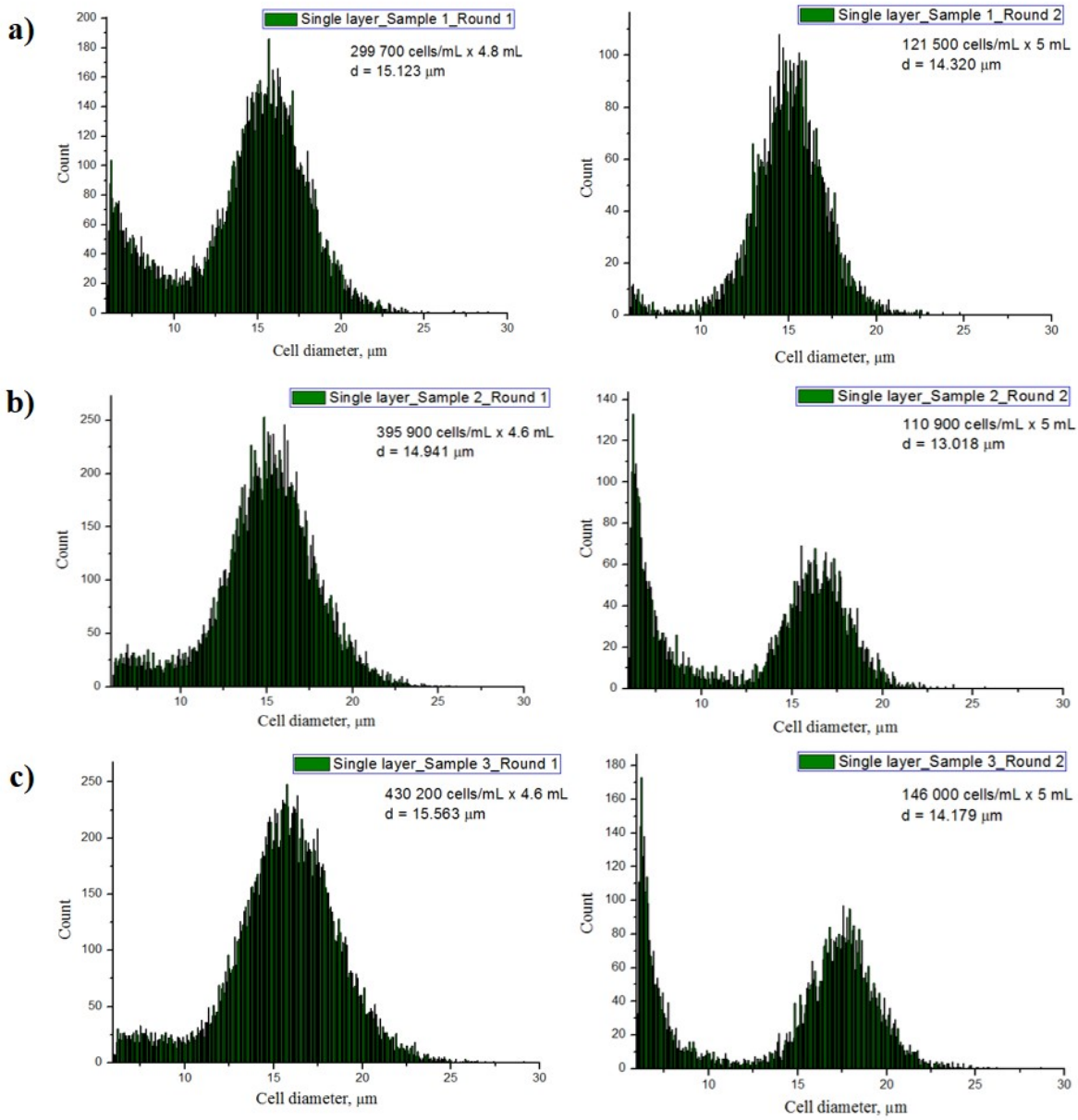


Figure S7. Cell diameter distribution histograms obtained for single-layer scaffold triplicates: (a) sample 1; (b) sample 2; and (c) sample 3

Table S1. Calculation of fiber surface area for a single fibrous layer

Vertical position of bundles along circular scaffold, mm	Fiber bundle length, mm	Single fiber diameter, mm	Surface area of 8 cylinders, mm^2	Total surface area, mm^2
-9.5000	0.0000	0.0019	0.0000	126.3348
-9.3929	2.8449		0.1358	
-9.2858	4.0119		0.1915	
-9.1787	4.8996		0.2338	
-9.0716	5.6413		0.2692	
-8.9645	6.2890		0.3002	
-8.8574	6.8692		0.3279	
-8.7503	7.3979		0.3531	
-8.6432	7.8855		0.3764	
-8.5361	8.3391		0.3980	
-8.4290	8.7640		0.4183	
-8.3219	9.1643		0.4374	
-8.2148	9.5430		0.4555	
-8.1077	9.9026		0.4726	
-8.0006	10.2451		0.4890	
-7.8935	10.5722		0.5046	
-7.7864	10.8852		0.5195	
-7.6793	11.1854		0.5339	
-7.5722	11.4738		0.5476	
-7.4651	11.7511		0.5609	
-7.3580	12.0183		0.5736	
-7.2509	12.2759		0.5859	
-7.1438	12.5246		0.5978	
-7.0367	12.7648		0.6092	
-6.9296	12.9970		0.6203	
-6.8225	13.2217		0.6310	
-6.7154	13.4393		0.6414	
-6.6083	13.6500		0.6515	
-6.5012	13.8542		0.6612	
-6.3941	14.0521		0.6707	
-6.2870	14.2441		0.6798	
-6.1799	14.4304		0.6887	
-6.0728	14.6111		0.6974	
-5.9657	14.7865		0.7057	
-5.8586	14.9568		0.7139	
-5.7515	15.1222		0.7218	
-5.6444	15.2828		0.7294	
-5.5373	15.4387		0.7369	
-5.4302	15.5901		0.7441	
-5.3231	15.7372		0.7511	

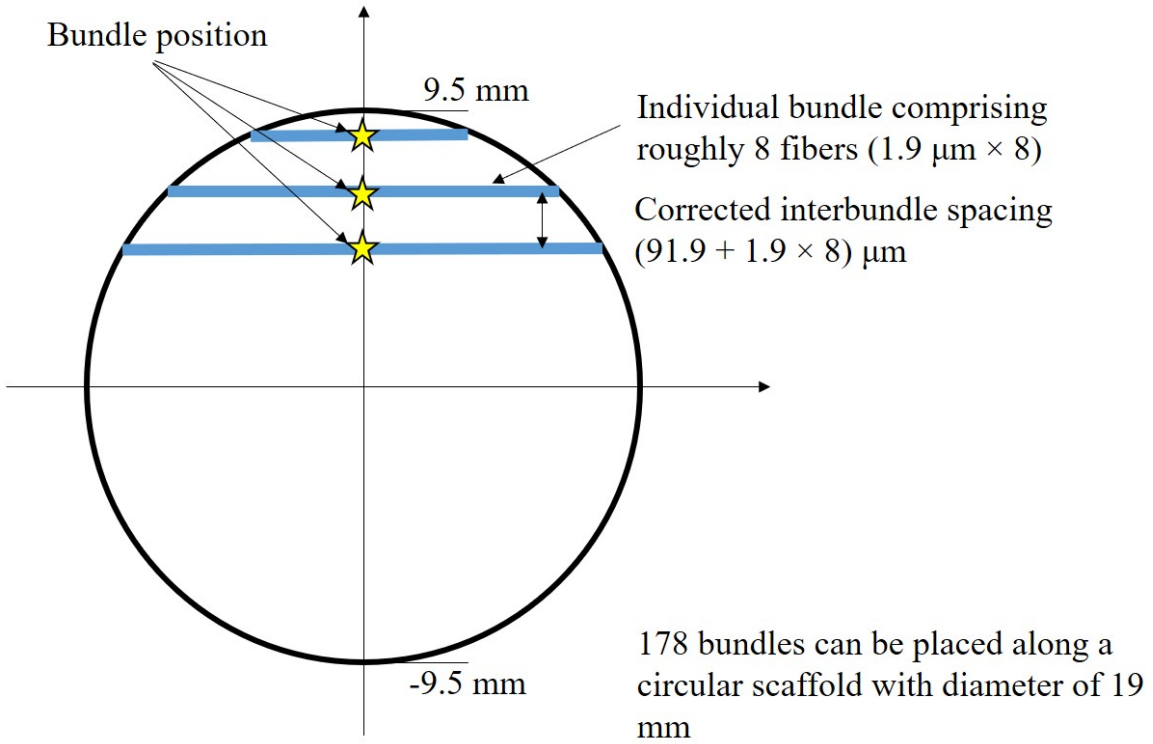
-5.2160	15.8800		0.7579	
-5.1089	16.0186		0.7645	
-5.0018	16.1533		0.7710	
-4.8947	16.2840		0.7772	
-4.7876	16.4108		0.7833	
-4.6805	16.5340		0.7891	
-4.5734	16.6534		0.7948	
-4.4663	16.7693		0.8004	
-4.3592	16.8816		0.8057	
-4.2521	16.9905		0.8109	
-4.1450	17.0961		0.8160	
-4.0379	17.1983		0.8208	
-3.9308	17.2973		0.8256	
-3.8237	17.3930		0.8301	
-3.7166	17.4856		0.8346	
-3.6095	17.5752		0.8388	
-3.5024	17.6616		0.8430	
-3.3953	17.7451		0.8469	
-3.2882	17.8256		0.8508	
-3.1811	17.9031		0.8545	
-3.0740	17.9778		0.8580	
-2.9669	18.0497		0.8615	
-2.8598	18.1187		0.8648	
-2.7527	18.1849		0.8679	
-2.6456	18.2484		0.8710	
-2.5385	18.3091		0.8739	
-2.4314	18.3672		0.8766	
-2.3243	18.4226		0.8793	
-2.2172	18.4753		0.8818	
-2.1101	18.5254		0.8842	
-2.0030	18.5729		0.8864	
-1.8959	18.6178		0.8886	
-1.7888	18.6601		0.8906	
-1.6817	18.6999		0.8925	
-1.5746	18.7372		0.8943	
-1.4675	18.7719		0.8959	
-1.3604	18.8042		0.8975	
-1.2533	18.8339		0.8989	
-1.1462	18.8612		0.9002	
-1.0391	18.8860		0.9014	
-0.9320	18.9083		0.9025	
-0.8249	18.9282		0.9034	
-0.7178	18.9457		0.9042	
-0.6107	18.9607		0.9050	

-0.5036	18.9733		0.9056	
-0.3965	18.9834		0.9060	
-0.2894	18.9912		0.9064	
-0.1823	18.9965		0.9067	
-0.0752	18.9994		0.9068	
0.0319	18.9999		0.9068	
0.1390	18.9980		0.9067	
0.2461	18.9936		0.9065	
0.3532	18.9869		0.9062	
0.4603	18.9777		0.9058	
0.5674	18.9661		0.9052	
0.6745	18.9520		0.9045	
0.7816	18.9356		0.9038	
0.8887	18.9167		0.9029	
0.9958	18.8953		0.9018	
1.1029	18.8715		0.9007	
1.2100	18.8453		0.8994	
1.3171	18.8165		0.8981	
1.4242	18.7853		0.8966	
1.5313	18.7515		0.8950	
1.6384	18.7153		0.8932	
1.7455	18.6765		0.8914	
1.8526	18.6352		0.8894	
1.9597	18.5914		0.8873	
2.0668	18.5449		0.8851	
2.1739	18.4959		0.8828	
2.2810	18.4442		0.8803	
2.3881	18.3899		0.8777	
2.4952	18.3329		0.8750	
2.6023	18.2733		0.8721	
2.7094	18.2109		0.8692	
2.8165	18.1458		0.8661	
2.9236	18.0779		0.8628	
3.0307	18.0072		0.8594	
3.1378	17.9337		0.8559	
3.2449	17.8573		0.8523	
3.3520	17.7780		0.8485	
3.4591	17.6957		0.8446	
3.5662	17.6105		0.8405	
3.6733	17.5222		0.8363	
3.7804	17.4308		0.8319	
3.8875	17.3364		0.8274	
3.9946	17.2387		0.8228	
4.1017	17.1378		0.8180	

4.2088	17.0336		0.8130	
4.3159	16.9261		0.8078	
4.4230	16.8151		0.8026	
4.5301	16.7007		0.7971	
4.6372	16.5827		0.7915	
4.7443	16.4611		0.7857	
4.8514	16.3357		0.7797	
4.9585	16.2066		0.7735	
5.0656	16.0735		0.7672	
5.1727	15.9365		0.7606	
5.2798	15.7954		0.7539	
5.3869	15.6501		0.7469	
5.4940	15.5004		0.7398	
5.6011	15.3464		0.7325	
5.7082	15.1877		0.7249	
5.8153	15.0243		0.7171	
5.9224	14.8560		0.7090	
6.0295	14.6827		0.7008	
6.1366	14.5041		0.6923	
6.2437	14.3201		0.6835	
6.3508	14.1304		0.6744	
6.4579	13.9349		0.6651	
6.5650	13.7333		0.6555	
6.6721	13.5252		0.6455	
6.7792	13.3105		0.6353	
6.8863	13.0888		0.6247	
6.9934	12.8596		0.6138	
7.1005	12.6227		0.6025	
7.2076	12.3775		0.5908	
7.3147	12.1236		0.5786	
7.4218	11.8603		0.5661	
7.5289	11.5872		0.5530	
7.6360	11.3034		0.5395	
7.7431	11.0081		0.5254	
7.8502	10.7003		0.5107	
7.9573	10.3791		0.4954	
8.0644	10.0430		0.4793	
8.1715	9.6905		0.4625	
8.2786	9.3198		0.4448	
8.3857	8.9286		0.4261	
8.4928	8.5141		0.4064	
8.5999	8.0726		0.3853	
8.7070	7.5995		0.3627	
8.8141	7.0885		0.3383	

8.9212	6.5306		0.3117	
9.0283	5.9126		0.2822	
9.1354	5.2132		0.2488	
9.2425	4.3937		0.2097	
9.3496	3.3675		0.1607	
9.4567	1.8120		0.0865	

Variations in fiber length within an individual bundle are assumed to be negligible. The schematic shown below provides a conceptual framework supporting the preceding calculations.



Scheme S1. Schematic representation of the bundle position within a planar scaffold and the associated calculation concept