Implementation of stratified approach and gene immobilization to enhance the osseointegration of silk-based ligament graft

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A

	Rp[µm]	Rv[µm]	Rz[µm]	Rc[µm]	Rt[µm]	Ra[µm]	Rq[µm]	Rsk	Rku	Rsm[µm]	RΔq[°]	Rδc[µm]
1	48.537	41.106	89.643	53.532	256.683	15.709	20.490	0.338	3.826	22.051	373.537	27.246
2	40.812	40.826	81.638	49.879	199.290	14.563	18.980	-0.045	3.694	26.354	359.741	26.483
3	49.889	40.290	90.179	67.152	403.290	14.924	20.290	0.194	4.361	31.276	376.715	25.736
4	41.328	35.645	76.974	47.050	313.896	13.455	17.888	0.162	3.941	27.422	342.252	22.416
5	39.332	38.759	78.091	41.873	236.525	12.566	16.927	-0.018	4.679	25.977	316.822	20.681
6	29.621	24.279	53.900	34.720	123.967	10.123	12.901	0.256	3.138	22.806	241.480	18.947
7	23.709	25.396	49.105	31.865	187.611	7.515	10.306	-0.024	4.463	25.855	226.858	13.219
8	31.054	29.339	60.392	37.419	173.853	11.230	14.448	0.209	3.449	24.421	272.077	17.810
9	22.169	22.178	44.347	29.594	133.927	7.143	9.689	0.043	4.296	25.228	202.492	12.053
10	39.436	32.224	71.661	45.215	399.297	11.785	15.973	0.074	3.949	28.646	296.964	14.356
	10	10	10	10	10	10	10	10	10	10	10	10
AVG	36.589	33.004	69.593	43.830	242.834	11.901	15.789	0.119	3.980	26.004	300.894	19.895
MIN	22.169	22.178	44.347	29.594	123.967	7.143	9.689	-0.045	3.138	22.051	202.492	12.053
MAX	49.889	41.106	90.179	67.152	403.290	15.709	20.490	0.338	4.679	31.276	376.715	27.246
σ	9.595	7.343	16.633	11.356	100.666	2.964	3.883	0.132	0.479	2.706	63.240	5.583
3σ	28.785	22.029	49.899	34.069	301.997	8.893	11.649	0.395	1.437	8.117	189.719	16.749

B

	Rp[µm]	Rv[µm]	Rz[µm]	Rc[µm]	Rt[µm]	Ra[µm]	Rq[µm]	Rsk	Rku	Rsm[µm]	RΔq[°]	Rδc[µm]
1	76.912	91.333	168.245	112.454	544.136	27.881	37.370	-0.054	4.542	28.685	737.063	46.735
2	86.966	77.365	164.332	102.896	630.473	26.211	35.370	0.266	4.562	25.573	712.251	44.540
3	91.999	106.385	198.385	125.334	510.872	38.237	48.214	-0.218	3.709	27.977	882.001	70.046
4	101.160	109.209	210.369	132.953	572.271	38.959	50.161	0.051	3.952	25.788	977.717	62.497
5	87.918	90.009	177.928	113.682	565.725	31.763	40.634	-0.030	3.825	28.438	771.357	56.063
6	86.539	97.462	184.001	113.762	666.083	31.487	41.610	-0.126	4.283	26.458	831.362	56.499
7	92.886	103.168	196.055	117.028	582.867	32.351	43.565	-0.053	4.146	27.773	843.626	56.366
8	96.397	108.014	204.410	135.104	489.661	38.227	49.456	-0.086	3.538	32.344	894.598	63.272
9	87.786	100.415	188.201	116.371	476.227	34.314	43.481	-0.228	3.976	27.125	865.176	62.175
10	61.705	61.808	123.513	64.727	290.724	19.879	26.811	0.033	3.984	23.991	542.884	34.701
	10	10	10	10	10	10	10	10	10	10	10	10
AVG	87.027	94.517	181.544	113.431	532.904	31.931	41.667	-0.045	4.052	27.415	805.804	55.289
MIN	61.705	61.808	123.513	64.727	290.724	19.879	26.811	-0.228	3.538	23.991	542.884	34.701
MAX	101.160	109.209	210.369	135.104	666.083	38.959	50.161	0.266	4.562	32.344	977.717	70.046
σ	11.014	15.099	25.281	19.715	103.669	6.048	7.168	0.143	0.336	2.265	121.401	10.523
3σ	33.041	45.296	75.842	59.146	311.006	18.145	21.505	0.429	1.008	6.794	364.204	31.570

Figure S1: Analysis of representative "Ra" parameter for roughness of silk-based scaffold with ligament (A) and bone (B) regions.
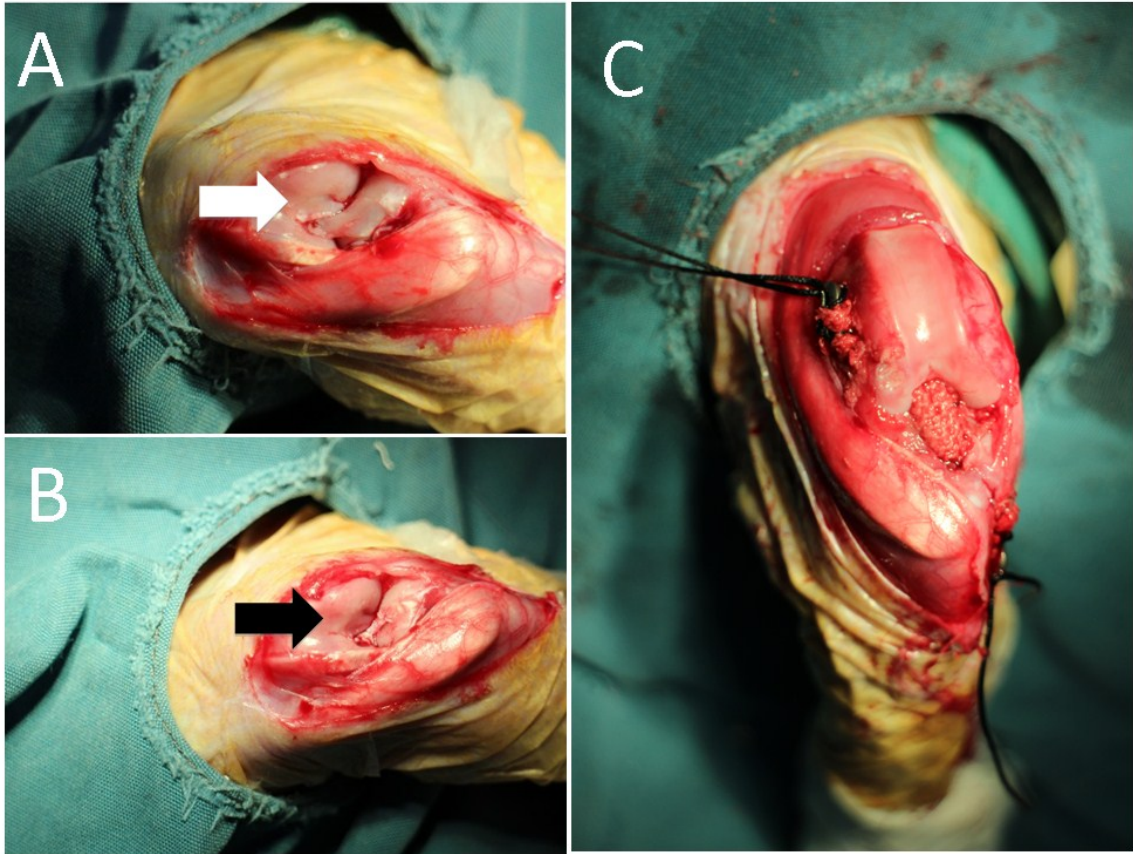
Figure S2: The silk-based graft implanted into rabbit ACL-defect model. (A) The rabbit ACL was exposed after opening the knee joint cavity (White arrow indicates ACL); (B) the removed ACL was prepped for graft implantation (Black arrow indicates the removed ACL). (C) Silk-based graft was placed on the ACL-defect with fixation to bone tunnels positioned within femur and tibia.
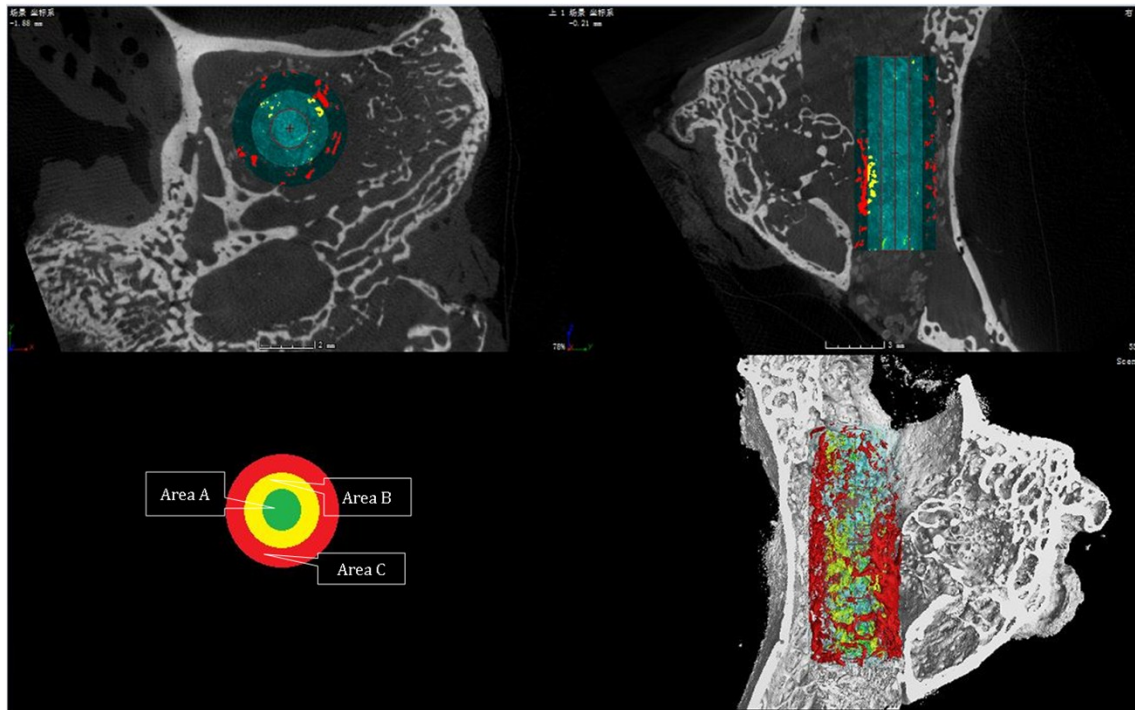
Figure S3: Three-dimensional construction image of microCT scan for evaluating osseointegration regeneration of silk-based graft *in vivo*.

Table S1: Primer sequences for real-time PCR assay.

Genes	Primer (Forward)	Primer (Reverse)
COL1A2	GCATGTCTGGTTAGGAGAAACC	ATGTATGCAATGCTGTTCTTGC
COL2A1	AAGAGCGGTGACTACTGGATAG	TGCTGTCTCCATAGCTGAAGT
Tenascin-C	TCTCTGCACATAGTGAAAAACAATACC	TCAAGGCAGTGGTGTCTGTGA
Runx2	CCTTCCACTCTCAGTAAGAAGA	CCTTCCACTCTCAGTAAGAAGA
Sox9	CTTCATGAAGATGACCGACGAG	CTCTTCGCTCTCCTTCTTGAGG
TGF-β3	TGGCTGTTGAGAAGAGAGTCC	TGCTTCAGGGTTCAGAGTGTT
BMP2	CGCAGCTTCCATCACGAAG	GTGGTCCACCGCATCACAG
GAPDH	GACATCAAGAAGGTGGTGAAGC	CTTCACAAAGTGGTCATTGAGG

Table S2: Mechanics property of silk scaffold（n=6, $\overline{x}$ ±s, *P＜0.05）.

Sample	Maximum tensile load（N）	Stiffness（N/mm）	Maximum tensile displacement（mm）
Rabbit ACL	131.82±17.64	47.07±14.84	3.01±1.03
Silk graft	169.11±9.75*	65.46±8.90*	2.87±0.56

Table S3: Maximum pullout force examined for silk-based graft (Femur and Tibia sides) derived from Rabbit（n=6, $\overline{x}$ ±s）.

Group	4W（N）	8W（N）	12W（N）
Femur (E)	14.38±3.27	20.45±4.78	41.95±8.87
Femur (C)	5.64±4.98	16.57±6.31	26.81±6.19
Tibia (E)	13.20±4.96	25.72±11.76	41.18±8.85
Tibia (C)	7.23±4.50	15.04±5.08	27.54±5.63

E, Experimental group; C, Control group.