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1 **Mesenchymal stem cell interacted with PLCL braided scaffold coated with**  
2 **Poly-L-lysine/Hyaluronic Acid for Ligament tissue engineering**

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10 **Abstract:** The challenge of finding an adapted scaffold for ligament tissue  
11 engineering still remains unsolved after years of researches. A technology to fabricate  
12 a multilayer braided scaffold with flexible and elastic Poly (L-lactide-co-caprolactone)  
13 (PLCL 85/15) has been recently pioneered by our team. In the present study,  
14 polyelectrolyte multilayer films (PEM) with poly-L-lysine (PLL)/ hyaluronic acid  
15 (HA) were deposited on this scaffold. After PEM modification, polygonal (PLL) and  
16 particle-like (HA) structures were present on the braided scaffold with no significant  
17 variation of fibers Young's modulus. Wharton's Jelly Mesenchymal Stem Cells  
18 (WJ-MSC) and Bone Marrow Mesenchymal Stem Cells (BM-MSC) showed good  
19 metabolic activity on scaffolds. They presented a spindled shape along the fiber  
20 longitudinal direction, and crossed the fibers to form cell bridges. Collagen type I,  
21 collagen type III and tenascin-C secreted by MSCs were detected on day 14.  
22 Moreover, one-layer modified scaffold presented increased chemotaxis. As a  
23 conclusion, our results indicate that this braided PLCL scaffold with one layer PEM  
24 modification shows inspiring potential with satisfying mechanical properties and

1 biocompatibility. It opens new perspectives to incorporate growth factors within  
2 PEM-modified braided PLCL scaffold for ligament tissue engineering and to recruit  
3 endogenous cells after implantation.

4 **Keyword:** Poly (L-lactide-co-caprolactone), braided scaffold, mesenchymal stem cell,  
5 polyelectrolyte multilayer films, ligament tissue engineering

## 6 **Introduction**

7 Ligament injuries are among the highest incidence of musculoskeletal disorders,<sup>1-4</sup>  
8 especially for the people who commonly do sports, rigorous physical activities, suffer  
9 acute trauma, or for aged people. The main strategy for repairing injured ligament  
10 consists in a surgical intervention called ligamentoplasty. Grafts<sup>5-7</sup> used in such  
11 interventions include autografts, allografts, and artificial substitutes. These therapies  
12 are subject to constant improvements,<sup>5,8</sup> resulting in the increase of joint stability,  
13 recovered mechanical properties, and patients' overall satisfaction. However, such  
14 surgeries are still associated with drawbacks<sup>9,10</sup> including donor site morbidity,  
15 immune rejection, or limited graft sources. With the rise of tissue engineering,  
16 ligament tissue engineering could constitute a novel, attractive and alternative  
17 approach to recover the function of injured ligament.<sup>1, 3, 7</sup>

18 Scaffolds with the architectures of sponges, knitted fibers, meshes, hydrogels, wired  
19 yarns, and braided fibers have already been reported<sup>3, 11, 12</sup> in ligament tissue  
20 engineering. Despite their benefits, they also suffer from some drawbacks,<sup>3, 12, 13</sup> such

1 as insufficient mechanical properties, brittle behavior, or lack of functional groups for  
2 molecular signaling. In our previous studies,<sup>14, 15</sup> PLCL has been used to develop a  
3 novel multilayer braided scaffold. It presents a porous structure for cell or tissue  
4 ingrowth, nutrient exchange, and post-graft degradation. Thanks to the adjustable  
5 geometrical parameters of this architecture (e.g. number of layers, fiber diameter and  
6 braiding angle), the mechanical strength could also be adjusted to fulfill requirements  
7 of different ligaments. PLCL is a synthetic biodegradable co-polymer with high  
8 elasticity consisting of polylactide (PLLA) and polycaprolactone (PCL). It has already  
9 been pointed out<sup>16</sup> to support tendon cell proliferation, and has been widely applied in  
10 tissue engineering because of its good biocompatibility and mechanical properties.

11 Seeding cells<sup>17-19</sup> could also contribute to the functionality of tissue engineered  
12 constructs by encouraging its colonization and extra cellular matrix (ECM) synthesis.

13 Ligament fibroblasts<sup>19</sup> may appear as natural candidate reparative cells for ligament  
14 tissue engineering, but there are limited by cell availability, quantity of donors and  
15 also differentiation capacity. Because of self-renew ability, differentiation potential,  
16 and bioactive molecule secretion activity, Mesenchymal Stem Cells (MSCs) have  
17 become a popular cell source applied in tissue engineering.<sup>17, 20</sup> In addition, MSCs  
18 have already been reported<sup>17, 20</sup> to proliferate better and produce more collagen than  
19 fibroblasts. Wharton's Jelly Mesenchymal Stem Cells (WJ-MSC) and Bone Marrow  
20 Mesenchymal Stem Cells (BM-MSC) were thus selected as cell sources in our present  
21 study.

1 In the last decades, Layer-By-Layer (LBL) technology has been widely used to  
2 modify the surface of biomaterials in order to improve the integration or add  
3 specialized properties to scaffolds.<sup>21, 22</sup> Examples of LBL biological applications  
4 include mediation of cellular functions to promote cell adhesion, proliferation,  
5 differentiation; biosensors; drugs or bioactive molecular delivery system, and  
6 biomimetics. The cellular adhesion and homogeneous cell distribution on the scaffold  
7 is a primary element in tissue engineering, and many studies<sup>7, 23</sup> have emphasized the  
8 importance of growth factors to promote MSCs attachment, proliferation, and matrix  
9 synthesis in the process of ligament/tendon repair. In the light of the advantages of  
10 LBL technology for cell adhesion, and the demand of growth factor delivery system  
11 for tissue engineering, LBL modification was introduced in our study in order to  
12 promote a ligamentous tissue. Poly-L-lysine (PLL) and Hyaluronic Acid (HA) were  
13 selected<sup>24, 25</sup> as polycation and polyanion for PEM. HA, as a component of  
14 extracellular matrix, plays an important role in improving cell growth, differentiation  
15 and migration, but it has been observed to be non-adhesive to cells.<sup>24, 26</sup> On the  
16 contrary, PLL has been shown<sup>27, 28</sup> to promote cell adhesion and it has widely been  
17 used for biomaterial coating and drug delivery. The pair of PLL/HA PEM was  
18 expected to be an effective system to improve MSCs behavior on scaffolds.

19 Prior to any incorporation of growth factors into a LBL-treated scaffold, it is crucial  
20 to study the effect of PLL/HA LBL modification on the behaviors of WJ-MSC and  
21 BM-MSC on PLCL braided scaffold. Therefore, in the present contribution the effect

1 of LBL technology on MSCs proliferation, distribution, morphology, collagen  
2 synthesis, tenascin-C expression and migration were evaluated, as well the  
3 consequence of this treatment on scaffold topology, and mechanical properties.

#### 4 **METHODS AND MATERIALS**

##### 5 **Scaffold fabrication**

6 PLCL (85/15) braided scaffold was fabricated according to previously described  
7 methods.<sup>14, 15</sup> Briefly, it consists of a multilayer braided structure, each layer being  
8 made up of 16 fibers of poly(lactide-co- $\epsilon$ -caprolactone) (PLCL, Purac Biomaterials)  
9 with a lactic acid/ $\epsilon$ -caprolactone proportion of 85/15. The polymer was firstly  
10 processed into 170  $\mu$ m fibers using a homemade extrusion machine at 180°C. Fibers  
11 were collected on bobbins, and 16 bobbins were installed on a maypole braiding  
12 machine (Composite & Wire Machinery, United States) to form braided scaffolds. Six  
13 layers were superposed in total, for a resulting scaffold of 96 fibers measuring around  
14 4mm in diameter.

##### 15 **Scaffold modification with LBL technology**

16 PLL (Sigma) and HA (Acros) were dissolved in the NaCl-Tris buffer (pH6.3) at a  
17 concentration of 1 mg/mL respectively. Scaffolds were cut into 1 cm-long parts. Firstly,  
18 scaffolds were immersed into PLL solutions for 7 min, then gently rinsed in NaCl-Tris  
19 buffer for 1 min. They were subsequently introduced into HA solution for 7 min, and  
20 then rinsed. Scaffold-blank (SB) refers to the scaffold without LBL modification;  
21 scaffold-PLL (SP) refers to scaffold modified only by PLL solution, and  
22 scaffold-PLL-HA-PLL (S1L) refers to one-layer modification. This configuration

1 (pH6.3 and only one-layer modification) was selected based on preliminary studies  
2 briefly reported in supplementary material.

### 3 **Scaffold characterization**

#### 4 **Characterization of scaffold by Fourier Transform Infrared Spectroscopy (FTIR)**

5 PLCL fibers were vacuum-dried at 60°C for 24 h, and cut into fine powders for the  
6 measurements by FTIR. The dry powders were mixed with Potassium bromide (KBr),  
7 and spectra were recorded on an FTIR spectrometer (Nicolet, USA) over the  
8 wavenumber range from 4000 to 600 cm<sup>-1</sup>.

#### 9 **Detecting of PLL/HA coating by Atomic Force Microscopy (AFM) and Confocal**

#### 10 **Laser Scanning Microscopy (CLSM)**

11 The surface of modified fibers was characterized by AFM (Oxford instruments). PLCL  
12 fibers were cut into 1 cm and maintained on a glass slide. Surface topology was  
13 observed and recorded in contact mode.

14 PLL-FITC (Sigma) was used to replace PLL in PLL/HA system. Fibers were  
15 immersed in PLL-FITC/HA solution using the procedure described above, then  
16 detected by CLSM (Nikon).

#### 17 **Scaffold morphology by Scanning Electronic Microscopy (SEM)**

18 Scaffolds were frozen with liquid nitrogen for 10 min, dried with vacuum oven for 7 h,  
19 then coated with gold and observed by SEM (TESCAN, Czech).

#### 20 **Mechanical properties of fibers**

21 The mechanical properties of PLCL fibers were assessed using tensile tests performed

1 with a Zwick Roell 2.5 device (Zwick, Germany), at a tensile speed of  $0.1\text{mm}\cdot\text{s}^{-1}$ . Six  
2 10 cm-long fibers were tested in parallel for SB, SP and S1L (n=4). The following  
3 loading cycle was prescribed 1) 1% strain then unloading 2) 3% strain then unloading 3)  
4 increasing strain up to failure. Young's modulus was measured for each sample from  
5 the slope of the stress-strain curve just after the first unloading cycle (at 1% of strain).

### 6 **Mesenchymal Stem Cell isolation and expansion**

7 Human bone marrow and human umbilical cords were supplied by CHRU Hospital and  
8 Maternity Hospital (Nancy, France) with the informed consent of donors. Firstly, 25 mL  
9  $\alpha$ -MEM (Lonza) medium containing 10% fetal calf serum (FCS, Dominique Dutscher),  
10 2 mM L-glutamine (Sigma), 100 U/mL penicillin /streptomycin (Gibco) and 1  $\mu\text{g}/\text{mL}$   
11 amphoterin B (Gibco) was added to 20 mL bone marrow, and centrifuged 300g for 5  
12 min. The supernatant was removed, and cells were seeded on tissue culture plates at the  
13 density of  $5\times 10^4$  cells/cm<sup>2</sup>, and then incubated in 37°C, 5% CO<sub>2</sub>, 90% humidity until  
14 the cells reach 70% - 80% confluence.

15 Human umbilical cord was washed with 70% ethanol and cut into 3-4 cm long samples  
16 in the HBSS buffer. The cord was opened to peel off two arteries and one vein; the jelly  
17 was torn off from cordon and cut into fine pieces in HBSS buffer. After 7 days, jelly  
18 was removed, and the culture medium was changed twice a week until the cells reach  
19 70% - 80% confluence. All MSCs used for assays were in passage 3 (P3). Before  
20 seeding cells on scaffolds, the quality of MSCs was controlled by testing biological  
21 properties as CFU test, senescence test and phenotype characterization. (data shown in



1 supplementary material)

## 2 **MSC culture on scaffold**

3 Firstly, scaffolds were immersed in ethanol for 30 min, then sterilized with UV for 30  
4 min. MSCs were distributed on scaffold ( $3 \times 10^5$  cells, 50  $\mu\text{L}$ /scaffold).  
5 MSC-scaffolds were kept in incubator for 30 min, then supplied with 0.95 mL  $\alpha$ -MEM  
6 complete medium including 100  $\mu\text{g}/\text{mL}$  ascorbic acid (Sigma) and cultured for two  
7 weeks.

## 8 **Evaluation of cell biocompatibility on the scaffolds**

### 9 **Cell metabolic activity of MSCs on scaffold**

10 Alamar Blue (AB) tests were performed on day 1, 3, 5, and 7. 1 mL 10% AB work  
11 solution (Thermo Fish scientific) was added to each scaffold, and incubated for 4 h.  
12 The absorbance was measured at 570 nm and 600 nm (n=3). Negative control was AB  
13 working. The percentage of Alamar Blue Reagent reduction was calculated according  
14 to manufacturer's instructions.

### 15 **Cell morphology and distribution on scaffold**

#### 16 **Scanning Electronic Microscopy (SEM)**

17 MSC-Scaffolds were firstly fixed with 2.5% glutaraldehyde at 4°C overnight on day 14,  
18 then washed with PBS three times. MSC-scaffolds were frozen with liquid nitrogen for  
19 10 min, dried with vacuum oven for 7 h, and then images were taken by SEM.

#### 20 **Confocal microscopy**

21 Firstly, MSC-scaffolds were fixed with 1% paraformaldehyde for 10 min at room

1 temperature, then washed with PBS three times. Permibilization treatment was  
2 performed by adding 1 mL 0.5% triton-PBS to each well for 20 min and washed with  
3 PBS three times. 250  $\mu$ L Alexa 488 Phalloidin (Life technologies) solution was  
4 supplied to each MSC-scaffold for 45 min, then washed with PBS three times. 200  $\mu$ L  
5 DAPI (1/2000) (Sigma) was added to each scaffold for 15 min, and washed with PBS  
6 three times. Images were taken by confocal microscopy (Leica SP5-CFS).  
7 Quantification of cell nuclear elongation was performed by Image J software version  
8 1.51k. The length was defined as the longest distance through nucleus and the width  
9 was defined as the distance perpendicular to the length. Cell nuclear aspect ratio was  
10 calculated by dividing the length to the width. The area of cell nucleus ( $n \geq 100$  from  
11 more than three images) was measured by Image J 1.51k.

### 12 **Extracellular matrix synthesized by MSCs on scaffold**

13 After 2 weeks, MSC-scaffolds were firstly fixed with 1% paraformaldehyde for 10 min,  
14 then washed with PBS three times. 5% PBS-BSA blocking buffer was added to  
15 scaffolds for 30 min. The first antibody anti-collagen type I (CALBIOCHEM),  
16 collagen type III (Sigma) and anti-tenascin-C (Abcam) were added (250  $\mu$ L) to  
17 scaffolds for 1 h, then washed with PBS three times. The second antibody anti-Rabbit  
18 IgG-Alexa488 (1/50) (Life technologies) and anti-mouse IgG-Alexa488 were  
19 distributed (250  $\mu$ L) to scaffold for 45 min, then washed with PBS three times. 200  $\mu$ L  
20 DAPI (1/2000) was added to each scaffold for 15 min. MSC-Scaffolds were washed  
21 with PBS three times, and observed by fluorescence microscopy. Negative controls

1 were performed as MSC with only second antibody, and positive controls were tested  
2 as fibroblast with anti-collagen I , collagen III and tenascin-c and second antibody  
3 ( data shown in supplementary material).

#### 4 **Cell Chemotactic assay**

5 PLCL films were fabricated by hot-processing method, and modified with PLL/HA  
6 PEM as PLCL braided scaffold treatment. Boyden chamber assay was performed for  
7 migration test by using a transwell (Corning® FluoroBlok™) with 0.8 μm pore  
8 fluorescence blocking PET track-etched membrane insert according to manufacturer's  
9 instructions. The downside of insert was coated with 100 μL collagen for 30 min.  
10 MSCs were starved 24 h before cell migration assay. SB, SP, and S1L film with 500  
11 μL α-MEM complete medium were introduced to each reservoir.  $1.5 \times 10^5$  cell  
12 suspension in α-MEM without FCS (200 μL) was added into each insert. After 24 h,  
13 cells were stained with calcein AM (Thermo Fish scientific) (1/1000) for 30 min, then  
14 washed with PBS three times. Cell distribution was observed by fluorescence  
15 microscopy and fluorescence intensity was measured by fluorescence  
16 spectrophotometer.

## 17 **RESULTS**

### 18 **Physiochemical properties of PLCL braided scaffold with/without PLL/HA** 19 **modification**

### 20 **Fourier-transform infrared spectroscopy of PLL/HA LBL modification**

1 The structure of PLCL fiber with/without PLL/HA modification are shown in Fig.1 by  
2 FTIR. The spectra at 2990  $\text{cm}^{-1}$  (PLLA), 2945  $\text{cm}^{-1}$  (PLLA) and 2860 (PCL)  $\text{cm}^{-1}$   
3 demonstrated<sup>29</sup> the presence of alkyl group of copolymer PLCL. Bands at 1130  $\text{cm}^{-1}$   
4 and 1065  $\text{cm}^{-1}$  corresponded to ester backbone of PLA in scaffold SP and S1L  
5 respectively.<sup>30</sup> The wavelength of PLL in SP was present as anion group<sup>31</sup> at 1600  $\text{cm}^{-1}$ .  
6 The wavelength of HA in PLL/HA-PLL was present as carboxylate anion group<sup>31</sup> at  
7 1616  $\text{cm}^{-1}$  and 1413  $\text{cm}^{-1}$ . The wavelength of amine groups of PLL<sup>31</sup> was shown at 1630  
8  $\text{cm}^{-1}$  in PLL/HA-PLL.

#### 9 **Topology of PLL/HA LBL modification.**

10 PLL-FITC was observed to cover the fiber homogeneously by CLSM (Fig.2a), and  
11 from SP to S1L the fluorescence intensity was increased. As shown in Fig.2b, the  
12 surface of fiber was globally flat and smooth. Irregular polygonal structures on the  
13 surface of SP were deduced to correspond to PLL molecules, while the “particles”  
14 structures on S1L were attributed to HA molecules by AFM.

#### 15 **Morphology of scaffold**

16 SEM images of PLCL braided scaffold in Fig.3a indicated a porous and braided  
17 structure, and this multilayer structure was not influenced by PLL/HA modification.  
18 For SB (scaffold without modification), the fibers kept the same texture from original  
19 fabrication, while for SP and S1L the fibers presented a relatively smooth surface after  
20 modification. The architecture of this multi-layer braided scaffold is illustrated in

1 Fig.3b: the scaffold was composed with six layers shown in six different colors (16  
2 braided fibers each layer).

### 3 **Mechanical properties**

4 Typical stress-strain curves as well as Young's modulus evolution are exhibited in Fig.4.  
5 Young's modulus of SB,SP and S1L were respectively  $1616 \pm 643$  MPa,  $1608 \pm 156$   
6 MPa and  $1758 \pm 470$  MPa. No significant changes in Young's modulus were therefore  
7 observed. The three types of fibers broke for a strain around 15-25% with no significant  
8 effect of surface modification.

### 9 **Biocompatibility of MSC-scaffold in vitro**

#### 10 **MSC metabolic activity on scaffold**

11 Cell metabolic activity was detected by AB and is presented in Fig.5. The metabolic  
12 activity of WJ-MSC on SB, SP and S1L increased of 24.2%, 19.64%, and 12.82%  
13 respectively from day 1 to day 7. For BM-MSC, the activity increased of 21%, 15.77%  
14 and 15.95% respectively on SB, SP and S1L from day 1 to day 7. Although cell  
15 metabolic activities were improved both for WJ-MSC and BM-MSC from day 1 to  
16 day 7, the cell metabolic activities decreased as the surface treatment went from SB to  
17 SP and S1L, especially for WJ-MSC.

#### 18 **MSC morphology and location on the scaffold**

19 The morphology of MSCs on the scaffold by SEM (Fig.6) showed that cells gathered to  
20 form cell sheets between fibers, and also indicated ECM synthesis. Fluorescent images  
21 (Fig.7) displayed a spindle and elongated shape of MSCs along the fibers. MSCs

1 tended to grow along the fiber direction in a homogeneous manner, migrated and  
2 proliferated across fibers to form cell bridges. Quantification of morphological changes  
3 of nucleus induced by scaffolds is illustrated in Fig.8. Mean nuclear area of WJ-MSC  
4 ( $224.7 \mu\text{m}^2$ ) was smaller ( $p < 0.05$ ) than BM-MSC ( $246.9 \mu\text{m}^2$ ) on tissue culture  
5 polystyrene (TCPS). For WJ-MSC, the mean nuclear area did not change between  
6 cells on scaffold and cells on TCPS. In contrast, for BM-MSC, the mean nuclear area  
7 decreased for cells on SB and S1L compared to cell nucleus on TCPS. The nuclear  
8 aspect ratio (length/width) of cells significantly increased ( $p < 0.05$ ) comparing to cell  
9 nucleus on TCPS for both WJ-MSC and BM-MSC.

#### 10 **Extracellular matrix expression by MSC on the scaffold**

11 Results concerning collagen and tenascin-C expression are shown in Fig.9. Green  
12 points on the scaffold correspond to collagen type I, collagen type III and tenascin-C  
13 synthesized by WJ-MSC and BM-MSC respectively on day 14. It was observed that  
14 both types of collagens and tenascin-C were substantially secreted, independently of  
15 the surface modification.

#### 16 **Chemotactic properties of scaffold on MSC**

17 Cell migration was performed to evaluate the effect of scaffold and scaffold  
18 modification on MSCs chemotaxis. As observed on fluorescent images showed in  
19 Fig.10, the number of migrated BM-MSC tended to be higher than WJ-MSC induced  
20 by SB, SP and S1L, and this was confirmed by fluorescent intensity. It also  
21 demonstrated that MSCs chemotaxis was most activated under the stimulation of S1L

1 for WJ-MSC and BM-MSC by fluorescent spectrophotometer.

## 2 **DISCUSSION**

3 PLCL<sup>31</sup> is an elastomeric synthetic copolymer combining biocompatible, degradable  
4 PLLA and PCL, resulting in desirable mechanical properties. It is of great interest in  
5 tissue engineering,<sup>14,32</sup> because of its biocompatibility, biodegradability, and  
6 easy-processing. Vuornos et al<sup>33</sup> compared a foamed PLCL(70/30) structure with a  
7 braided poly(L/D)lactide (PLA, 96L/4D) scaffold for tendon tissue engineering, and  
8 PLCL was judged to be excessively elastic to form a braided structure in their study. On  
9 the contrary, our group developed a controllable method to fabricate a uniform, porous,  
10 braided structure based on elastic PLCL (85/15). Noteworthy, the number of fiber  
11 layers could be adjusted to fulfill the mechanical requirements of different ligaments.  
12 Moreover, PLL/HA layer-by-layer system was reported to be one of the classical PEM  
13 for cell attachment, proliferation and ECM synthesis in many works.<sup>24, 25</sup> In our study,  
14 we introduced PLL/HA PEM on a braided PLCL scaffold for the first time and  
15 confirmed by FTIR that PLL and HA were deposited on the surface of PLCL fibers<sup>29, 30,</sup>  
16 <sup>34</sup>. After the replacement of PLL by PLL-FITC, the fluorescence intensity was  
17 increased from SP to S1L by CLSM. A polygonal and particle-like structure of PLL and  
18 HA were additionally detected on scaffold by AFM.  
19 The interaction between cells and biomaterials is influenced<sup>35</sup> by physicochemical  
20 properties of scaffold (surface topography, surface toughness, wettability, stiffness, and  
21 crystallinity), and also cell sources. PLL/HA modification could therefore influence the

1 scaffold surface microenvironment<sup>36</sup> as local mechanical, wettability, toughness and so  
2 on. In a non-reported preliminary study, we firstly compared the deposition order of  
3 PLL and HA by testing cell metabolic activity, and the results demonstrated that PLL  
4 constitutes a cell-adhesive layer while HA is cell-repellent layer. PLL/HA  
5 polyelectrolyte films have been previously reported<sup>36</sup> to be a pH-responsive PEM, and  
6 both pH6.0-6.5 and pH7.4 have been applied for PLL/HA bilayer deposition prior to  
7 the present study<sup>24, 36</sup>. Based on these published data, the effects of pH (pH6.3 and  
8 pH7.4) on PEM deposition was assessed and different PLL/HA bilayers numbers were  
9 compared (reported in supplementary material) in order to optimize LBL modification  
10 on scaffold for both WJ-MSC and BM-MSC. As a result, for both MSCs, cell  
11 proliferation did not increase over one layer modification. Moreover, pH 6.3 for PEM  
12 deposition greatly encouraged MSCs metabolic activity, especially for WJ-MSC  
13 (reported in supplementary material). Consequently, in the present contribution, SB,  
14 SP and S1L made in pH6.3 were selected.

15 Cell attachment, proliferation, ECM synthesis and migration are a series of  
16 prerequisite activities for tissue regeneration. It is also modulated by bioactive  
17 molecular such as growth factors. In the present study, MSCs attached well on  
18 scaffolds as shown by the formation of cell sheets. There was no significant difference  
19 of cell attachment between non-modified and modified scaffolds. Cell cytoskeleton  
20 organization has been previously reported<sup>37</sup> to relate to cell fate on scaffold, to be  
21 beneficial to predict cell commitment, and it also indicates cell senescence. Senescent



1 mesenchymal stem cells were characterized with enlarged, irregular morphology,  
2 following with the decrease of phenotype and differentiation abilities<sup>38</sup>. Fluorescent  
3 images reported in the present study presented a spindle-shape cell cytoskeleton  
4 covering the fibers. The nuclear size and shape were also related to the physiological  
5 process<sup>39</sup> as cell senescence, differentiation, and diseases. The quantitative data of cell  
6 nuclear morphology indicated an elongated and relatively spindle-like nuclear shape  
7 with decreased nuclear area while increased aspect ratio for WJ-MS C and BM-MS C  
8 on scaffold than on TCPS. Cells grew along the longitudinal direction of the fibers  
9 which could be an adaptable, resistant cell-scaffold system to response to mechanical  
10 stimulation. The results of Alamar Blue assay indicated that cell proliferated well on  
11 scaffold, and the metabolic activity did not show significant change after PLL/HA  
12 scaffold modification for WJ-MS C or for BM-MS C. Collagen constitutes the main  
13 component of native ligament tissue<sup>40</sup>, and its high expression is a basic requirement  
14 for the formation of ligament-like extracellular matrix. In addition, Tenascin-C has  
15 been shown to be a promising specific indicator<sup>41</sup> of ligament ECM, and it has been  
16 reported<sup>42</sup> to be highly expressed in regenerative process of musculoskeletal tissue,  
17 and to provide elasticity to tissues in reaction to heavy tensile loading. In the present  
18 study, Collagen I, collagen III and tenascin-C were secreted by BM-MS C and  
19 WJ-MS C on the scaffold within 14 days, which could have been encouraged by  
20 ascorbic acid in the medium. This pointed out that BM-MS C-scaffold and  
21 WJ-MS C-scaffold had potential in the future to form ligamentous tissue. Additionally,

1 other specific indicators as tenomodulin and scleraxis could have been characterized,  
2 especially during a long-term culture including mechanical or biological stimulation.  
3 Cell migration is a crucial step in the tissue healing process since cells firstly migrate  
4 to the injury site or target area. Consequently, migration was assessed in present study  
5 and our results showed that chemotaxis of WJ-MSC and BM-MSC was activated by  
6 SB, SP and S1L. This migration was even more pronounced for one layer scaffold,  
7 compared to SB and SP. This may show a possibility to recruit MSC for *in vivo*  
8 implantation. These cell-scaffold constructs showed satisfying biocompatibility, and  
9 the effect of growth factors incorporation into this PEM layer will be assessed in the  
10 forthcoming developments.

## 11 **CONCLUSION**

12 A PLCL braided scaffold with PLL/HA modification was designed in our study. The  
13 mechanical, morphological and biological results concerning the activity of WJ-MSC  
14 and BM-MSC and the ECM synthesis showed that it may constitute a promising  
15 scaffold for ligament tissue engineering. The incorporation of LBL increased cell  
16 chemotaxis, which opens interesting perspectives for *in vivo* application. In the future,  
17 the developed LBL technology will be used to incorporate growth factors on PLCL  
18 braided scaffold in order to encourage tissue growth and the regeneration of a  
19 functional ligamentous tissue.

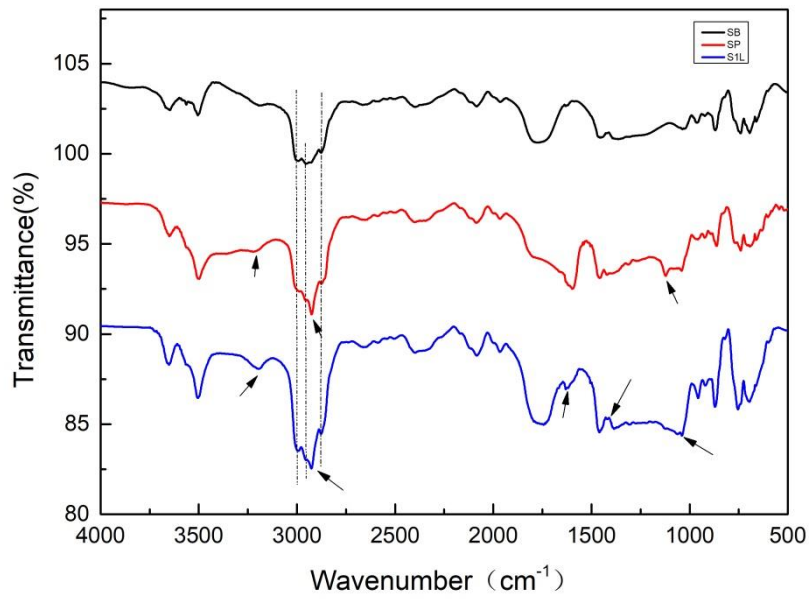
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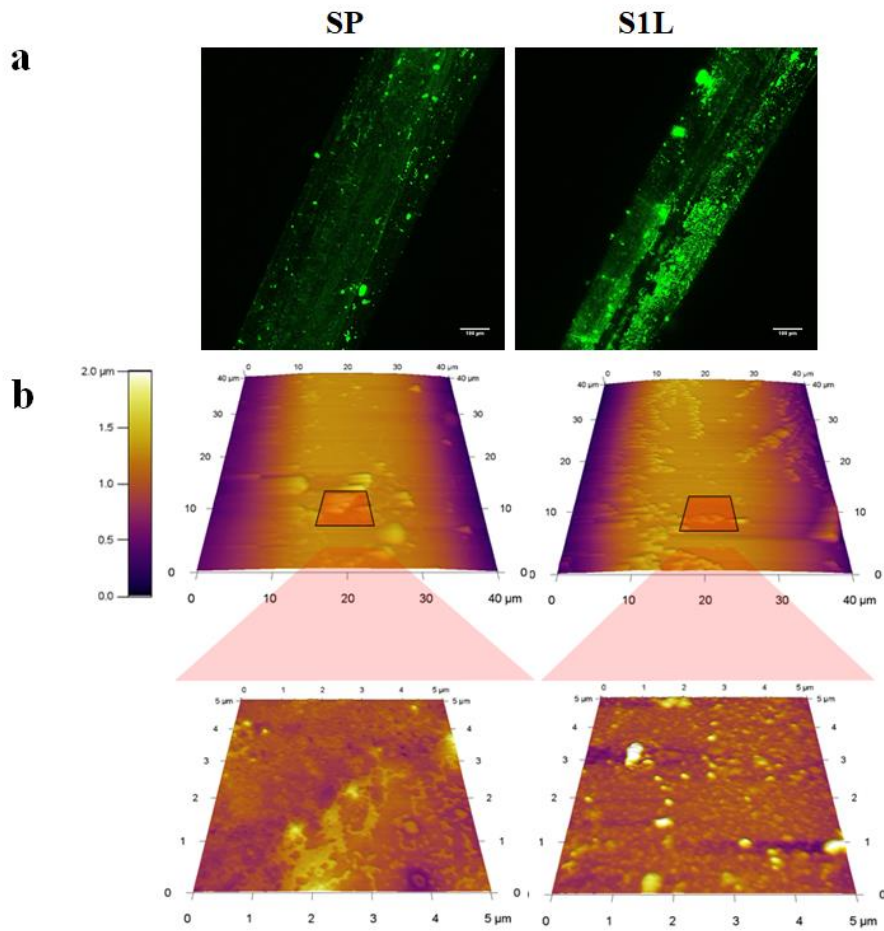
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2 Fig1. FTIR of PLL/HA modified on PLCL scaffold (SB:PLCL scaffold blank, SP:  
3 PLCL-PLL, S1L: PLCL-PLL/HA-PLL).

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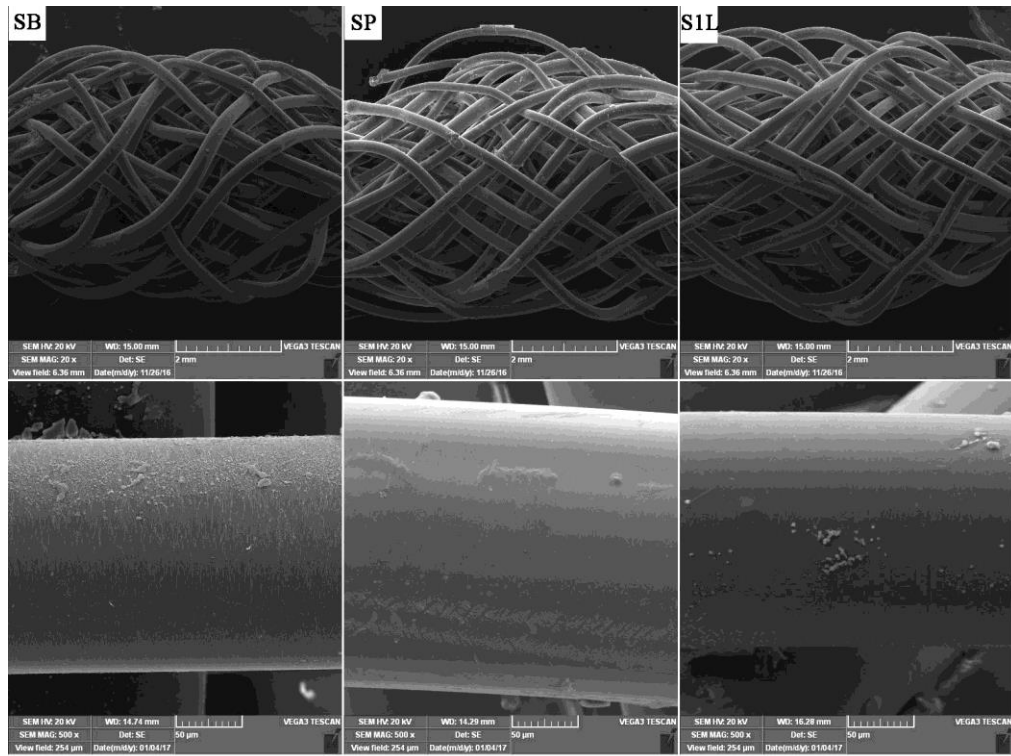


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2 Fig2. Morphology of PLL-FITC/HA on PLCL fiber by CLSM (a), and topology of  
 3 PLL/HA on PLCL fiber by AFM (b).(SP: PLCL scaffold-PLL-FITC, S1L: PLCL  
 4 scaffold-PLL/HA-PLL).

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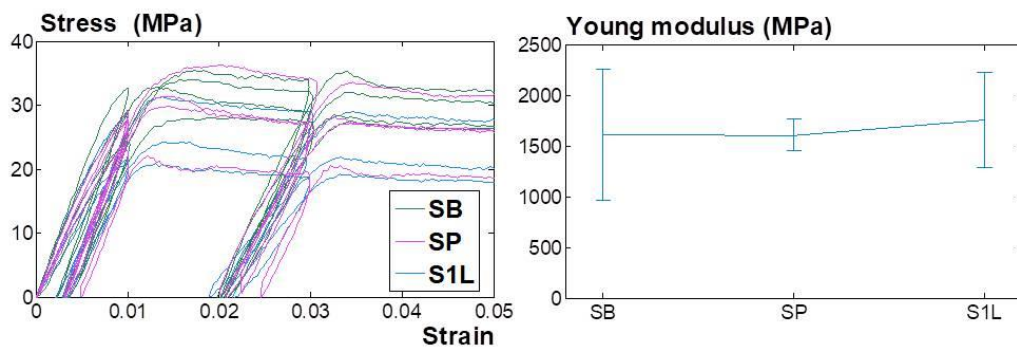
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2 Fig3. Morphology of PLCL scaffold and PLCL scaffold modified with PLL/HA by

3 SEM. (SB:PLCL scaffold blank, SP: PLCL-PLL, S1L: PLCL-PLL/HA-PLL).

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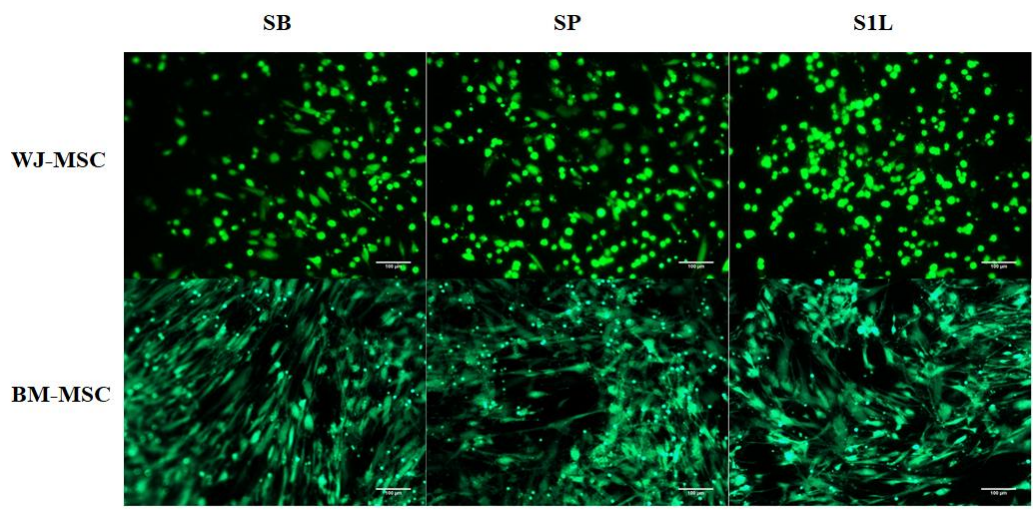
7 Fig4. Mechanical test of PLCL fibers (SB:PLCL fiber blank, SP: PLCL fiber-PLL, S1L:

8 PLCL fiber-PLL/HA-PLL).

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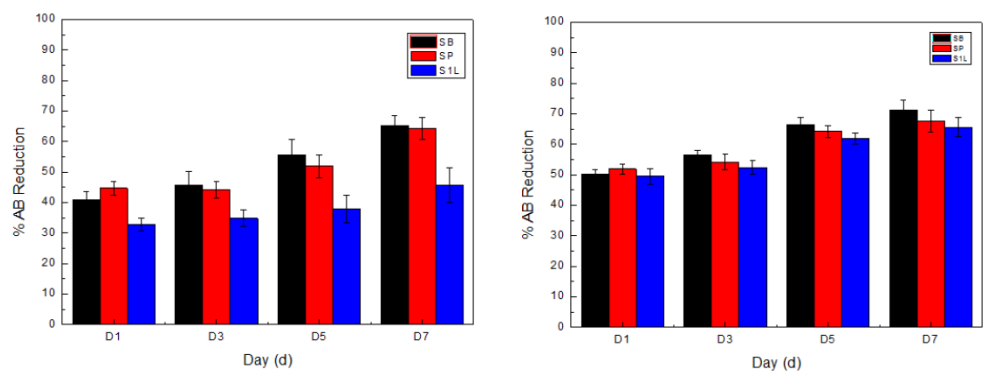


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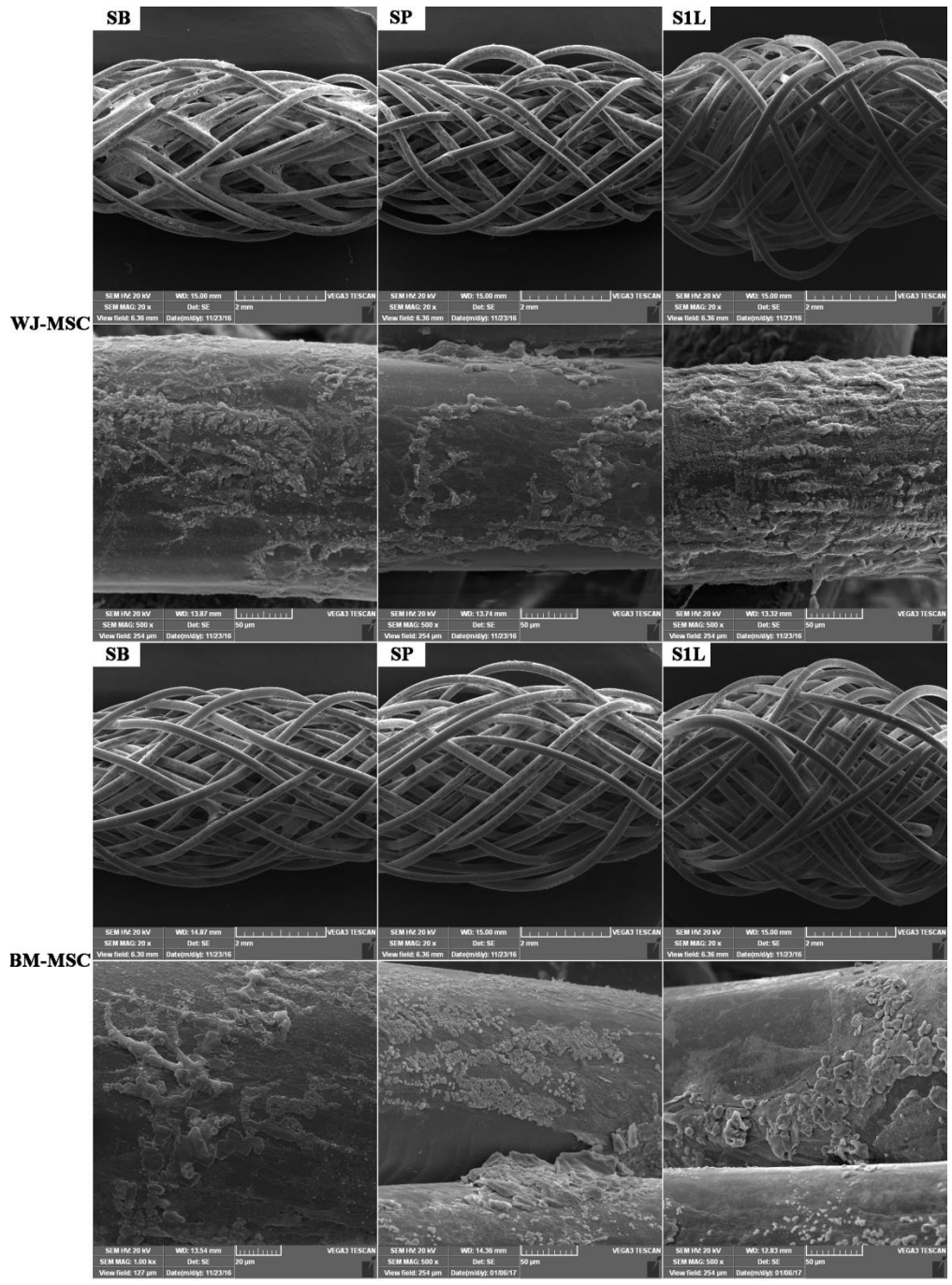
11 Fig5. Migration of WJ-MSC and BM-MSC induced by PLCL scaffold, scaffold-PLL  
12 and scaffold- PLL/HA-PLL.



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2 Fig6. Reduction of AB of WJ-MSC and BM-MSC on PLCL scaffold (SB:PLCL

3 scaffold blank, SP: PLCL-PLL, S1L: PLCL-PLL/HA-PLL).



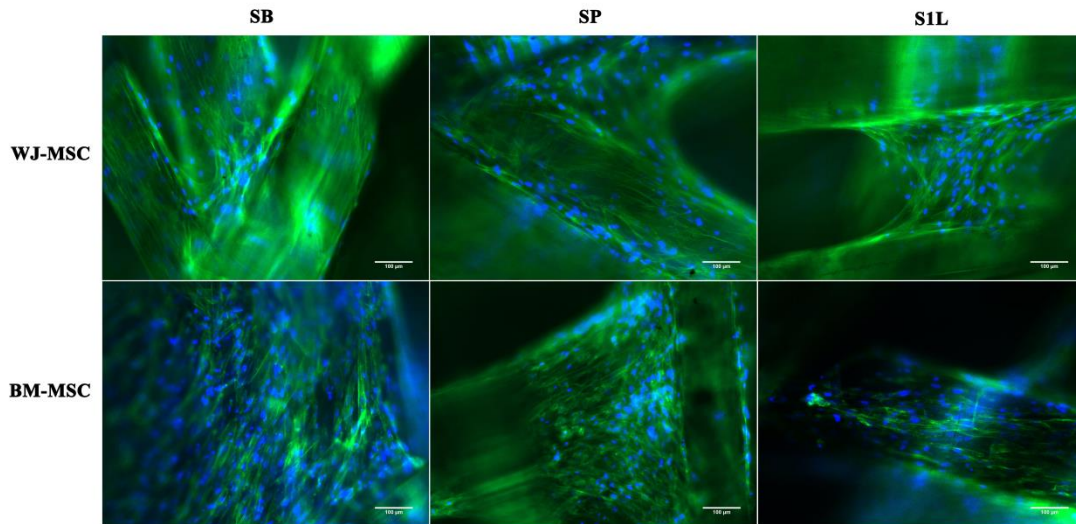
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2 Fig7. Morphology and distribution of WJ-MSC and BM-MSC on scaffold by SEM.

3 (SB:PLCL scaffold blank, SP: PLCL-PLL, S1L: PLCL-PLL/HA-PLL).

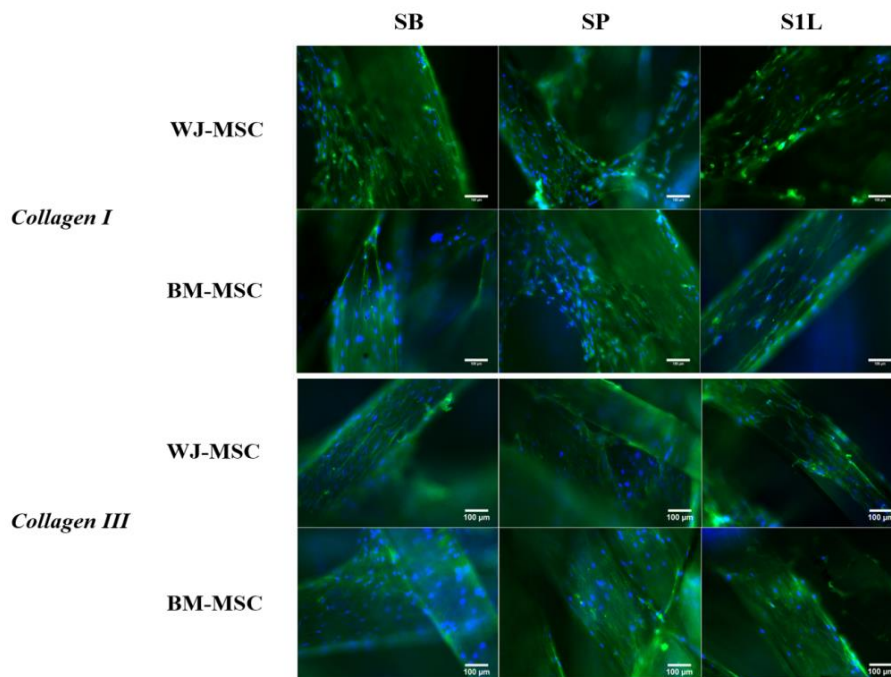
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2 Fig8. Morphology and distribution of WJ-MSC and BM-MSC on scaffold by  
 3 florescence microscopy.(SB: PLCL scaffold blank, SP: PLCL-PLL, S1L:  
 4 PLCL-PLL/HA-PLL).



5

6 Fig9. Collagen I and collagen III expression by WJ-MSC and BM-MSC on PLCL  
 7 scaffolds on D14. (SB:PLCL scaffold blank, SP: PLCL-PLL, S1L:  
 8 PLCL-PLL/HA-PLL).