Adhesion of Chondrocytes on Platelet-rich Plasma Pre-treated PLGA Mesh with Different Methods

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Introduction: Effective cell delivery to defect site is very important to enhance tissue regeneration. Especially, polymer biomaterials were widely applied for effective cell delivery due to its biocompatibility and biodegradability. PLGA (poly lactic-co-glycolic acid) is commercially available synthetic polymer which approved by FDA. Mesh type biomaterials showed thin and flexible property with large surface area. Therefore, PLGA mesh was applied as a chondrocytes carrier to meniscal tear lesion and it showed very good results in 15 of 17 menisci after implantation in vivo [1]. Platelet-rich plasma (PRP) is well known as cocktail with various growth factors including PDGF, TGFβ-1, IGF-1 and BMPs, and it is free from immune rejection since it is obtained from own blood of patient. Previous studies showed enhanced cell adhesion by addition of PRP into culture medium [2]. Therefore, PRP can be considered as a candidate for substitutable coating materials for enhancement of cell behaviors on synthetic biomaterials.

We hypothesized that PRP pretreatment with different methods on PLGA mesh would provide different patterns of platelet adhesion and activation stages, and finally would modulates cell adhesion and proliferation on the surface of PLGA mesh. In this study, we pretreated PLGA with PRP using three different methods (simple dripping, dynamic oscillation and centrifugation) and observed the amount of adhered platelets and their activation stage distribution. We further determined the adhesion of chondrocytes on PRP-pretreated PLGA meshes with different preparing method to investigate the effect of PRP amount and stage distribution on adhesion of chondrocytes.

Methods: PRP and scaffold preparation: allogeneic leukocyte-depleted PRP was obtained from our hospital blood bank. The mean platelet count in the PRP ranged from 1,000/nl to 1,300/nl. PRP of 5 donors was mixed and used for the experiments. The woven PLGA mesh scaffold (Vicryl™, Ethicon) measuring 20×8 mm was prepared and divided into 3 treatment groups. (1) Simple dripping method: The scaffolds were immersed into 1,000 μl of PRP in 6-well plates. Following 70 min of contact time in static conditions, the scaffolds were turned over and the same procedure was done for the other side. (2) Dynamic oscillating method: The scaffolds were placed in 15 ml polypropylene tubes containing 5 ml of PRP. The tubes were placed horizontally into a bidirectional rotator at 37 °c at an oscillation rate of 40 hz for 70 min. (3) Centrifugal method: The scaffolds were inserted into the bottom of 6 well plates and immersed into 1,000 μl of PRP. The plates were centrifuged at 150g for 10 min and incubated at 37 °c at an oscillation rate of 70 hz for 60 min. Then, the scaffold was flipped 180° and the same procedure was done for the other side of scaffolds.

Calcium treatment: After washing three times with DPBS (Dulbecco’s phosphate-buffered saline), half of the scaffolds were soaked into 1,000 μl of DMEM media at 37 °c for 10 min. The calcium in the media initiated the clotting cascade to form a fibrin network.

Platelet counting and categorization with stage: After pretreatment of PRP on PLGA mesh, each mesh was fixed and observed by scanning electron microscope (SEM). Number of adhered platelets on PLGA meshes was manually counted from SEM images with same area (1263 x 974 μm) and calculated the average densities of the adhered platelets per 10,000 square μm. And then the adhered platelets were classified according to the scheme of Ko et al. [3] and Frank et al. [4] which composed five stages of adhered morphology of platelets including round, dendritic, spread dendritic, spread, and fully spread. Chondrocytes isolation and culture: Human articular chondrocytes were isolated from normal femoral heads of 10 patients (45-68 years old) who underwent total hip arthroplasties. Full-thickness cartilage was minced and digested at 37°C with serial treatment of protease and collagenase. Isolated chondrocytes were cultured in monolayer with DMEM with 10% FBS, 100 units/mL of penicillin-streptomycin and 25mg/mL of L-ascorbic acid.

Analysis of chondrocytes adhesion: Prepared PRP pretreated PLGA meshes were placed in 24well culture plates and chondrocytes were seeded on each types of PLGA mesh. And then, culture plates were placed on bidirectional rotator with 40Hz of oscillation at 37°C during 24 hrs for homogenous distribution of cells. After 24 hrs, cell adhesion was determined using CCK-8 cell counting kit (Dojindo, Tokyo, Japan) as manufacturer’s instruction.

Statistics: the data was presented as average ± standard deviation and were compared via Kruskal-Wallis test. Differences were considered as significant for p values < 0.05.

Results: Adhesion of platelets was highest on PRP pretreated PLGA mesh via centrifugal method and calcium addition enhanced platelet adhesion on same types of meshes (Figure 1). Upon the addition of the culture media, the calcium in the media triggered the polymerization of fibrin producing a thin coating of fibrin network over the surface and within the interstices of the
scaffolds. Most of platelets on PRP pretreated mesh without calcium addition showed dendritic or spread-dendritic morphology. On the other hands, the portion of spread or fully spread platelets increased on PRP pretreated mesh with calcium addition (Figure 1).

Adhesion of chondrocytes on each types of PRP pre-treated meshes were analyzed after 24 hrs of culture. Highest cell adhesion was demonstrated on PLGA mesh pretreated with PRP using centrifugation. Cell adhesion was more enhanced by calcium addition. Contrary to these findings, calcium addition had no effect on cell adhesion on PLGA mesh pretreated with PRP using simple dripping or dynamic oscillation methods (Figure 2).

Discussion: PRP pretreatment on PLGA mesh by centrifugal method occurred large employment of platelets on the surface of PLGA mesh than other treatment methods. Calcium addition to PRP pretreated PLGA mesh increased platelet activation as well as initial adhesion of chondrocytes on centrifugal PRP pretreated PLGA mesh. These results demonstrated that the PRP pretreatment of PLGA mesh by centrifugation with calcium addition can be chosen as surface modification method for polymeric biomaterials which showed enhanced initial cell adhesion via regulation of platelet employment and activation on the surface of PLGA mesh.

Significance: Our results showed that the surface modification technique on PLGA mesh using PRP can be applied into tissue engineered approach for meniscal tear lesions of the avascular zone.

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Figure 1. Cumulative number of adhered platelets with the different stages on each types of PRP pre-treated meshes.
Figure 2. Number of adhered human chondrocytes (HCs) on each type of PRP pre-treated meshes after 24 hrs of culture. (*p<0.05 versus number of adhered HCs on mesh+PRP groups, **p<0.05 versus number of adhered HCs on mesh+PRP+Ca groups)