The Effects of Age and Platelet-rich Plasma on the ACL Cell Viability and Collagen Production
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Introduction: Recently, there has been increased interest in repairing anterior cruciate ligament (ACL) ruptures using platelet-rich plasma (PRP) to promote regeneration and healing. However, the healing potential of the ACL following rupture varies significantly with the age of the patient at the time of injury, and the characteristics of patients who may be good candidates for the stimulating healing of the ligament have not yet been defined. To begin to address this, an in vitro study was designed to evaluate the influences of age and skeletal maturity on the ACL’s activities and its responses to PRP. We hypothesized that the effects of PRP on the cell viability and collagen production would be different for ACL fibroblasts obtained from skeletally immature, adolescent and adult animals.

Methods: Porcine fibroblast cells were harvested from the ACLs of fifteen Yucatan pigs in three age groups (skeletal immature: 6 to 10 mos; adolescent: 12 to 18 mos, and Adult: older than 3 years), with five pigs in each age group. Two types of scaffold were used in this study: (1) collagen hydrogel (COL) and (2) collagen – platelet-rich plasma composite (CPC) hydrogel, and thus 6 experimental groups were established: ACL cells from immature, adolescent and adult animals in (1) collagen hydrogel (COL groups) and (2) CPC hydrogel (CPC groups). In COL groups, type I acid soluble collagen hydrogel was neutralized to a pH of 7.4 using sodium bicarbonate, and the neutralized mixture was seeded with ACL cells at the density of 5x10⁵ cells/ml. In CPC groups, porcine platelet-rich plasma was added to the collagen hydrogel and the final density of platelet in the hydrogel was 314±10³/ml. For all the groups, the hydrogel-cell mixture was delivered to 3cm long semicircular molds with a small polyester mesh at each end to anchor the gels. Each construct was placed into a well of 6-well plate, warmed in a humidified 5%CO2/37°C incubator for 1 hour to achieve gelation, and then cultured with DMEM media (with 10% FBS and 1% antibiotics). Media was changed every three days during the culture period.

Cell viability of ACL cells cultured in collagen or CPC hydrogel was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay on day 14. The mRNA expression level of collagen type I and III in ACL cells was assessed by real-time quantitative RT-PCR on day 14. Total RNA was extracted from constructs using RNeasy mini kit (Quagen, CA) and was reverse-transcribed into cDNA using RETROscript Kit (Ambion, TX). Amplification was performed in ABI PRISM 7900 Sequence Detection System (Applied Biosystems, CA) using SYBRGreen PCR Master Mix Kit (Applied Biosystems, CA). The transcript level of target genes normalized to GAPDH was calculated using the 2^ΔΔCt formula.

Data were calculated as means ± standard deviation and analyzed using two-way analysis of variance, values of p < 0.05 were considered significant.

Results: Cell viability: When cultured in collagen hydrogel, the cell viability of the adult-COL group was significantly higher than that of the adolescent-COL group (p<0.05, Fig.1.). The MTT value was 0.40±0.067, 0.32±0.054 and 0.45±0.062 in the groups of immature-COL, adolescent-COL and adult-COL, respectively. The addition of PRP to the collagen hydrogel resulted in a significantly increased MTT value in all the three age groups (Immature-CPC, 0.65±0.091, p<0.05; Adolescent-CPC, 0.48±0.053, p<0.05; Adult-CPC, 0.62±0.072, p<0.05, Fig.1.). The MTT value of the groups of Immature-CPC and Adult-CPC was significantly higher than that of the Adolescent-CPC group (p<0.05), and no difference was found between these two groups (p<0.05).

Type I collagen mRNA: When cultured in collagen hydrogel, the Adolescent-COL group exhibited a significantly higher level (0.046±0.020) in collagen III mRNA than the other two age groups (p<0.05, Fig.2B). There was no significant difference between the groups of Immature-COL and Adult-COL (0.011±0.004 and 0.010±0.006, respectively; p>0.05). The addition of PRP to the collagen hydrogel also resulted in a significantly increased expression level of collagen III mRNA in immature (Immature-CPC, 0.123±0.063; p<0.05) and adolescent (Adolescent-CPC, 0.169±0.058; p<0.05) porcine ACL cells, a level which was over 1000% and 250% higher than the level in these two ACL cells cultured in collagen hydrogel (groups of Immature-COL and Adolescent-COL), respectively. However, the expression level in adult porcine ACL cells (Adult-CPC, 0.013±0.006) was only increased 30% by the addition of PRP (p<0.05). The expression level of collagen III mRNA of Immature-CPC and Adolescent-CPC was significantly higher than that of Adult-CPC (p<0.05), and no difference was found between these two groups.

Discussion: The results of the in vitro study suggest that skeletal maturity has significant effects on ACL cell’s activities and its response to PRP. When cultured in collagen hydrogel, ACL cells from adult pigs had higher cell viability than those from adolescent pigs, whereas the cells from adolescent pigs had a higher expression level of collagen type III mRNA than those from immature and adult pigs. PRP is known to play a crucial process in successful wound healing, and is likely to be useful in stimulating healing of tissues with an impaired ability to heal, like the ACL. In this study, the addition of PRP to collagen hydrogel resulted in a significantly increased cell viability in the cells from the pigs of all the three age groups, however, stimulation of collagen type I and III mRNA expression was only observed in the cells from immature and adolescent pigs. While additional studies are needed, data obtained from this study suggest the skeletal maturity may influence the repair capacity of ACL and the promoting effects of PRP on ACL healing.

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