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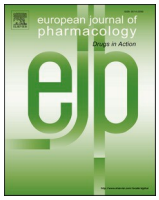

Young human PRP promotes the rejuvenation of aged bone marrow mesenchymal stem cells and the therapeutic effect on ischemic heart disease

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ABSTRACT

Bone marrow mesenchymal stem cell (BMSC) transplantation is an effective treatment for ischemic heart disease, but its effectiveness is limited in aging populations due to decreased viability and injury resistance of autologous BMSCs. The purpose of this study was to compare the differences between platelet-rich plasma (PRP) derived from young and aged donors, and to investigate whether it is possible to enhance the viability of elderly human BMSCs (hBMSCs) using PRP, and to apply the rejuvenated hBMSCs for the treatment of ischemia. The key growth factors in PRP, including IGF-1, EGF, and PDGF-BB, were found to have significant differences between young and old individuals. Our results showed that PRP could enhance the proliferation, cloning, and rejuvenation of aged hBMSCs, with a superior effect observed when using PRP derived from younger donors. In the SD rat infarct model, the application of hBMSCs optimized with PRP resulted in a smaller infarct area compared to the control group (NC-Old). Specifically, the infarct area in the group treated with hBMSCs cultured with PRP from young donors (YPRP-Old) was smaller than that in the group treated with PRP from older donors (OPRP-Old). The survival rate of hBMSCs after transplantation, the number of neovascularization in the infarct area of SD rats and the recovery of cardiac function were all higher in the YPRP-Old group than the OPRP-Old group, and both groups were better than the group treated with aged hBMSCs alone. In conclusion, PRP may provide a new stem cell transplantation therapy option for ischemic diseases.

1. Introduction

The incidence of cardiovascular diseases increases rapidly with age and has become a major cause of disability and death worldwide(Zhang et al., 2018; Roth et al., 2020). Ischemic heart diseases (IHDs), namely, coronary insufficiency, myocardial infarction (MI), and angina pectoris, are leading causes of death in the elderly population(Arnett et al., 2019). Ischemic injuries trigger irreversible myocardial necrosis and functional reduction of cardiomyocytes, severely compromising the contractile function of the heart. Despite advances in prevention and treatment of ischemic heart disease, none of the existing methods can effectively replenish cardiomyocytes due to the extreme difficulty of regenerating them, resulting in poor long-term cardiac function of patients(Gong et al., 2021).

Stem cell transplantation has emerged as a promising area of research for the treatment of ischemic heart disease, owing to the therapeutic benefits of self-renewal, multidirectional differentiation, and paracrine secretion, which are widely recognized(Yu et al., 2017). Among the various types of stem cells, BMSCs are one of the most commonly used stem cells (Goradel et al., 2018; Harrell et al., 2019; Fan et al., 2019). However, in clinical settings, hBMSCs used for transplantation therapy can only be derived from the patient's own cells to prevent rejection. Autologous stem cell transplantation often yields suboptimal results in elderly patients, as age is believed to be a critical factor(Dimmeler and Leri, 2008). Moreover, older stem cells have limited survival capacity and tissue regeneration potential following

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transplantation. Recent studies have demonstrated that the stemness and proliferation of stem cells can be regulated and enhanced(Li et al., 2019; Kong et al., 2019), and that genetic regulation of stem cells in older individuals can improve their resilience to injury and promote rejuvenation(Zhang et al., 2018; Zhang et al., 2020). In a study, young and old rats were successfully joined together by suturing their skin to create a model of shared blood flow, resulting in older animals exhibiting a youthful state and restoring a youthful phenotype(Scudellari, 2015). This finding suggests that the blood of younger animals may contain components capable of reversing aging and rejuvenating the organism. Blood can be centrifuged and concentrated to produce platelet-rich plasma (PRP), which is frequently used as a reservoir of natural growth factors due to its high concentration of these factors(Xu et al., 2020). PRP is easily prepared, does not cause rejections, and contains abundant growth factors in physiological proportions(Mehranfar et al., 2019; Hesseler and Shyam, 2019; Mao et al., 2019). Additionally, PRP has been clinically utilized to treat conditions such as carpal tunnel syndrome and lateral elbow pain(Hong et al., 2022; Karjalainen et al., 2021).

Based on the finding that blood from young rats can rejuvenate older animals and restore a youthful state, we hypothesize that platelet-rich plasma (PRP) derived from young blood may have anti-aging and rejuvenating effects on senescent cells. In this study, we compared the main growth factors present in PRP from young and old individuals, cultured aged human bone marrow-derived mesenchymal stem cells (hBMSCs) using PRP, and compared them in terms of proliferation, clastogenesis, senescence, and paracrine secretion. Furthermore, we evaluated the therapeutic potential of PRP-optimized hBMSCs in a rat model of ischemic heart disease.

2. Materials and methods

2.1. Extraction and culture of hBMSCs

All studies involving human participants were conducted in accordance with the ethical standards of the Research Ethics Committee of the Second Affiliated Hospital of Harbin Medical University and the principles outlined in the Declaration of Helsinki. Human bone marrow samples were obtained from patients (young age 18–30 years, old age 60–72 years) undergoing cardiac surgery at the hospital, with those suffering from other diseases being excluded. Informed consent was obtained from all patients prior to sample collection. The bone marrow was collected and transported to the laboratory within 2 h of collection. The BMSCs were isolated according to the previous method of our group (Bai et al., 2020). BMSCs were isolated from the bone marrow using a FicollPaque gradient (density of 1.073 g/mL; GE Healthcare, Little Chalfont, Buckinghamshire, UK) via centrifugation at 1330×*g* for 20 min. Single nucleated cells were collected, washed twice, and inoculated in a low-sugar essential medium (L-DMEM; HyClone, South Logan, Utah, USA) containing 10% fetal bovine serum (FBS; Sciencell, San Diego, CA, USA), before being cultured at 37 °C in 5% CO_2. The medium was replaced every two days, and third-generation BMSCs were used for the experiments.

2.2. Cell phenotyping

To determine the phenotype of young and old hBMSCs, we conducted flow cytometry analysis. The cells were stained with the following antibodies: CD34 (4ul, BioLegend, San Diego, CA, USA), CD45 (4ul, BioLegend), CD73 (4ul, BioLegend), CD90 (4ul, BioLegend), and CD105 (4ul, BioLegend). After incubating the cells at room temperature for 30 min, they were washed with cold phosphate buffered saline (PBS, pH7.4) three times. Immunoreactivity of the cell surface antibody markers was then detected using a flow cytometric fluorescence sorting technique (FACS, BD).

2.3. Preparation of PRP

The study was approved by the ethical review committee, and informed consent was obtained from all individuals from whom biological samples were collected. To prepare platelet-rich plasma (PRP), 30 ml of venous blood was drawn from 15 older donors aged 60–72 years and 12 younger donors aged 18–30 years. The blood was collected into a tube containing 3.8% (w/v) sodium citrate and centrifuged at 200×*g* for 10 min at 4 °C. The plasma layer above the red blood cells was carefully collected without disturbing the buffy layer and centrifuged at 1000×*g* for another 10 min. The upper 2/3 of the plasma was then aspirated to ensure consistency in the PRP concentration. Platelet count was measured using a complete blood counter (Mindray, Shenzhen, China) to ensure uniform platelet concentration in the PRP for each subgroup, which was calibrated to 1000×10^6/ml for each subgroup. To activate the plasma, 60u/ml of bovine thrombin (Sigma, St Louis, MO, USA) was added to obtain young PRP and old PRP.

2.4. PRP-related factor assay

Activated young and old PRPs were analyzed for six growth factors (GF) using an enzyme-linked immunosorbent assay (ELISA) kit specific for each GF (CUSABIO, Wuhan, Hubei, China). Transforming growth factor (TGF)-β1, epidermal growth factor (EGF), platelet-derived growth factor (PDGF)-BB, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and insulin-like growth factor (IGF)-1 were measured according to the manufacturer's recommendations.

2.5. Cell proliferation

To determine the optimal PRP concentration for aged cell growth, Cell Counting Kit-8 (CCK8; Dojindo, Kumamoto, Japan) was used according to the manufacturer's instructions. Cells were inoculated into 96-well plates (Corning Incorporated, Corning, NY, USA) at an initial density of 4000 cells per well. Different concentrations of PRP were received (in platelets/ml,10×10^6/ml, 50×10^6/ml, 100×10^6/ml and 200×10^6/ml). After 48 h of incubation, 100 μL of CCK8 reagent was added to each well. Cells were then incubated at 37 °C for 2 h. The absorbance of each well was measured at 450 nm using an enzyme marker (Tecan, Mannedorf, Switzerland).

The cells were divided into four groups: NC-young (young BMSCs, negative control), NC-old (old BMSCs, negative control), YPRP-old (old BMSCs supplemented with young PRP), and OPRP-old (old BMSCs supplemented with old PRP). The cells were seeded at 3×10^4 cells/well in 6-well plates (Corning). The experimental group was supplemented with 5% PRP/mL, while the control group was kept in a normal medium. After 48 h (Liu et al., 2014) of adding PRP, the medium was replaced with fresh medium, and the cells were counted at 3, 5, 7, and 9 days of culture using a cell counter (Bio-Rad, Hercules, CA, USA). The growth curves were plotted and compared.

2.6. Cell cloning

Young and old cells were seeded into six-well plates at a density of 800 cells per well. For the experimental group, the medium was supplemented with 5% PRP/mL, while the control group was kept in a normal medium. After treatment with PRP for 48 h, the medium was replaced with fresh medium every 3 days for the next 2 weeks. BMSCs were fixed with 4% paraformaldehyde (PFA) for 30 min and stained with crystal violet for 30 min. The colonies containing at least 50 cells were observed under a microscope and counted.

2.7. β-galactosidase staining

Cells were cultured in six-well plates, and grouped as described above. After treatment with PRP for 48 h, cells were analyzed for

senescence using an SA-β-gal staining kit (Beyotime, Shanghai, China). The cells were fixed with 4% PFA, washed with PBS, and incubated with SA-β-gal staining solution. The plates were then placed in a CO_2-free incubator at 37 °C overnight. The positive cells were stained blue, and the blue cells in the field of view was calculated.

2.8. Gene expression measurement

RNA was extracted from the subgroups 48 h after PRP treatment using TRIzol reagent (Life Technologies, Carlsbad, CA, USA). Reverse transcription was performed with the PrimeScript™ RT kit (Takara, Kusatsu, Shiga, Japan) according to the manufacturer's instructions. Real-time PCR was conducted on a thermal cycler (S1000; Bio-Rad) using FastStart™ Universal SYBR® Green premix (Rox) (Roche, Penzberg, Upper Bavaria, Germany). The Ki-67, p16, and p21 gene expression levels were determined with the following program: 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 20 s. The relative gene expression was calculated using the formula: relative gene expression $= 2^{-\Delta\Delta CT}$. Shanghai Biotech designed and synthesized the primers, which are listed in Table 1.

2.9. Westen blot

After 48h of treatment in the aforementioned groups, cells were lysed in RIPA (Beyotime) buffer containing protease inhibitors. Protein concentrations were quantified using a BCA kit (Beyotime) according to the manufacturer's instructions. Proteins were then separated by SDS-PAGE gels and transferred to PVDF membranes. Subsequently, membranes were incubated with the following primary antibodies: rabbit anti-human p16 (1:2000, Abcam, Cambridge, UK), rabbit anti-human p21 (1:5000, Abcam), and rabbit anti-human β-actin (1:1000, CST, Danvers, MA, USA). After this, the membranes were treated with goat anti-rabbit immunoglobulin G (IgG)-horseradish peroxidase (HRP) (1:1000, ZSGB-BIO, Beijing, China) secondary antibody at room temperature, followed by exposure to chemiluminescent substrate for development. Specific complexes were then photographed and analyzed using an automated chemiluminescence imaging analysis system (Tanon, Shanghai, China), according to the manufacturer's instructions.

2.10. Angiogenesis

After the intervention with PRP, the cell culture supernatant after 48h of replacing the new medium was resuspended with human umbilical vein endothelial cells (HUVECs, China), and the cells were seeded in 96-well cell culture plates coated with Matrigel matrix (BD Biosciences, San Jose, CA, USA). After incubation at 37 °C for 2h, capillary structures were observed under a microscope (Olympus, Japan) and the number of formed vessels was counted.

2.11. Cell immunofluorescence

The cells were seeded into 96-well plates (Corning), subjected to the aforementioned treatment groups, fixed with 4% PFA, permeabilized with 0.1% Triton X-100 (Beyotime), and then incubated with mouse anti-human Ki-67 antibody (1:1000, Abcam). Next, cells were incubated with TRITC-conjugated rabbit anti-mouse IgG (1:1000, CST) secondary antibody, and nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, Beyotime). Images were captured using a fluorescence microscope (Olympus, Japan), and the percentage of positive cells within the field of view was calculated.

Table 1
Primers used for real-time PCR.

Name	Sequence
P16	forward,5′-GGCCGATCCAGGTCATGATGATG-3′ reverse,5′-CACCAGCGTGTCCAGGAAGC-3′
P21	forward,5′-GATGGAACTTCGACTTTGTCAC-3′ reverse,5′-GTCCACATGGTCTTCCTCTG-3′
Ki-67	forward,5′-CAGACATCAGGAGAGACTACAC-3′ reverse,5′-GTTAGACTTGCTGCTGAGTCTA-3′
GAPDH	forward,5′-ACATCGCTCAGACACCATG-3′ reverse,5′-TGTAGTTGAGGTCAATGAAGGG-3′

2.12. MI model and cell transplantation

All animal procedures conducted in this study adhered to ethical standards outlined in the Guide for the Care and Use of Laboratory Animals and were approved by the Research Ethics Committee of the Second Affiliated Hospital of Harbin Medical University (SYDW2022-122). Adult male Sprague Dawley (SD) rats weighing between 200 and 220g were obtained from the Animal Experimentation Center of the Second Hospital of Harbin Medical University. Rats were injected intraperitoneally with cyclosporine A (5mg/kg; Novartis, Basel, Switzerland) daily for three days before surgery and throughout the experiment. The rats were placed in the supine position and tracheally intubated (arterial puncture needle, 16G) and anesthetized with isoflurane using a ventilator (Harvard Apparatus, Medford, NJ, USA). The left open chest was ligated with a 7–0 prolene needle 1.5mm below the left auricle to the anterior descending branch of the left coronary artery, and myocardial infarction was confirmed by the local color change of the myocardium. Ten minutes after myocardial infarction, cells (2.0×10^6/100 μL) were injected into one site in the center of the infarct area and four sites at the edge of the infarct area. Then, the incision was closed. Postoperative analgesics (buprenorphine, 0.1mg/kg body weight) were administered to the animals until the end of the experimental observation. The experiment was divided into five groups: culture medium group, NC-Young group, NC-Old group, YPRP-Old group, and OPRP-Old group.

2.13. Cardiac function assessment

Cardiac function was evaluated by echocardiography prior to myocardial infarction induction, as well as 1 and 4 weeks after the procedure. Under anesthesia, the animals were positioned on their left side, and M-mode echocardiographic images were obtained using a 12MHz transducer (EPIQ7; Philips, Amsterdam, New York, USA). Measurements of left ventricular end-diastolic internal diameter (LVEDd), left ventricular end-systolic internal diameter (LVESd), left ventricular ejection fraction (LVEF), and left ventricular short-axis shortening (LVFS) were taken.

2.14. Infarct size measurement

Four weeks after cell transplantation, the animals were anesthetized and euthanized by cervical dislocation. The chest was opened and the heart was removed. A custom-made balloon was inserted into the left ventricle through the mitral valve and sutured in place. The balloon was connected to a pressure detector and kept at 20 mmHg. Under these conditions, the heart was fixed in 4% PFA for 1 week and then sectioned. Masson's trichrome staining was used to assess collagen deposition. The entire section was scanned under a microscope, which allowed for the measurement of the size of the myocardial infarction (MI) on each of the myocardial surfaces. The final infarct size was expressed as an average percentage from sections of each ventricle.

2.15. Survival of transplanted cells in vivo

At 4 weeks after myocardial infarction and cell transplantation, rats were euthanized, and their hearts were fixed in 4% PFA for 24 h. The hearts were then dehydrated in sucrose solutions of 10%, 20%, and 30% for 1, 2, and 24 h, respectively. Tissues were embedded in special molds containing OCT compound (Sakura, Torrance, CA, USA). The frozen

tissue was cut into 5 μm thick sections and incubated with mouse anti-human mitochondrial antibody (1:200; Abcam), followed by incubation with goat anti-mouse IgG-TRITC (1:100; ZSGB-BIO). The sections were stained with DAPI (Beyotime) for cell nuclei. Positive cells (blue nuclei surrounded by red cytoplasm) were identified by immunofluorescence in five fields of view of each sample, and the results were tallied.

2.16. Infarct area angiogenesis

Four weeks after cell transplantation, frozen sections of hearts were prepared as described above. Immunofluorescence experiments were carried out by incubating the sections with rabbit anti-rat α-smooth muscle actin (α-SMA; 1:400; Affinity, Jiangsu, China) and goat anti-rabbit IgG-TRITC (1:1000, CST). The vessels in the area were then counted under light microscopy, and the results were averaged to determine the vessel density of microarteries and capillaries in the infarct margin area.

2.17. Statistical analysis

Statistical analysis was conducted using SPSS 23.0 (SPSS, USA) and GraphPad Prism 7 (GraphPad Software, CA, USA). Data were presented as mean ± standard deviation (SD), and comparisons between two groups were made using a two-tailed Student's t-test. ANOVA was employed to determine significance between three or more experimental groups. Statistical significance was indicated as $p < 0.05$.

3. Results

3.1. Characterization of hBMSCs

Young bone marrow mesenchymal stem cells (YBMSCs) and old bone marrow mesenchymal stem cells (OBMSCs) were isolated from young and aged human bone marrow using a gradient centrifugation method. The BMSCs obtained from the sternum were cultured up to the third generation, with YBMSCs displaying a spindle and fibroblastic morphology, and OBMSCs displaying an elongated flatter fibroblastic morphology (Fig. 1A). Flow cytometry analysis of BMSC-specific marker expression showed that both YBMSCs and OBMSCs were $CD34^{-}$, $CD45^{-}$ and $CD73^{+}$, $CD90^{+}$ and $CD105^{+}$ (Fig. 1B), suggesting that both samples were mesenchymal stem cells (expressing CD73, CD90 and CD105), and not hematopoietic stem cells (expressing CD34) or leukocytes (expressing CD45).

3.2. Comparison of the differences between young and old PRP major growth factors

The mean ages of young and old volunteers were 26 ± 3.54 years and 65.53 ± 3.85 years respectively. Leukocyte (P = 0.865), erythrocyte (P = 0.422) and platelet (P = 0.388) counts in whole blood did not differ significantly between these two groups in Table 2.

To determine the major growth factors in platelets, standardized PRP (1000 × 10^6/ml) were lysed. The analysis showed no significant differences in TGF-β1 (P = 0.192), FGF (P = 0.069), and VEGF (P = 0.274)

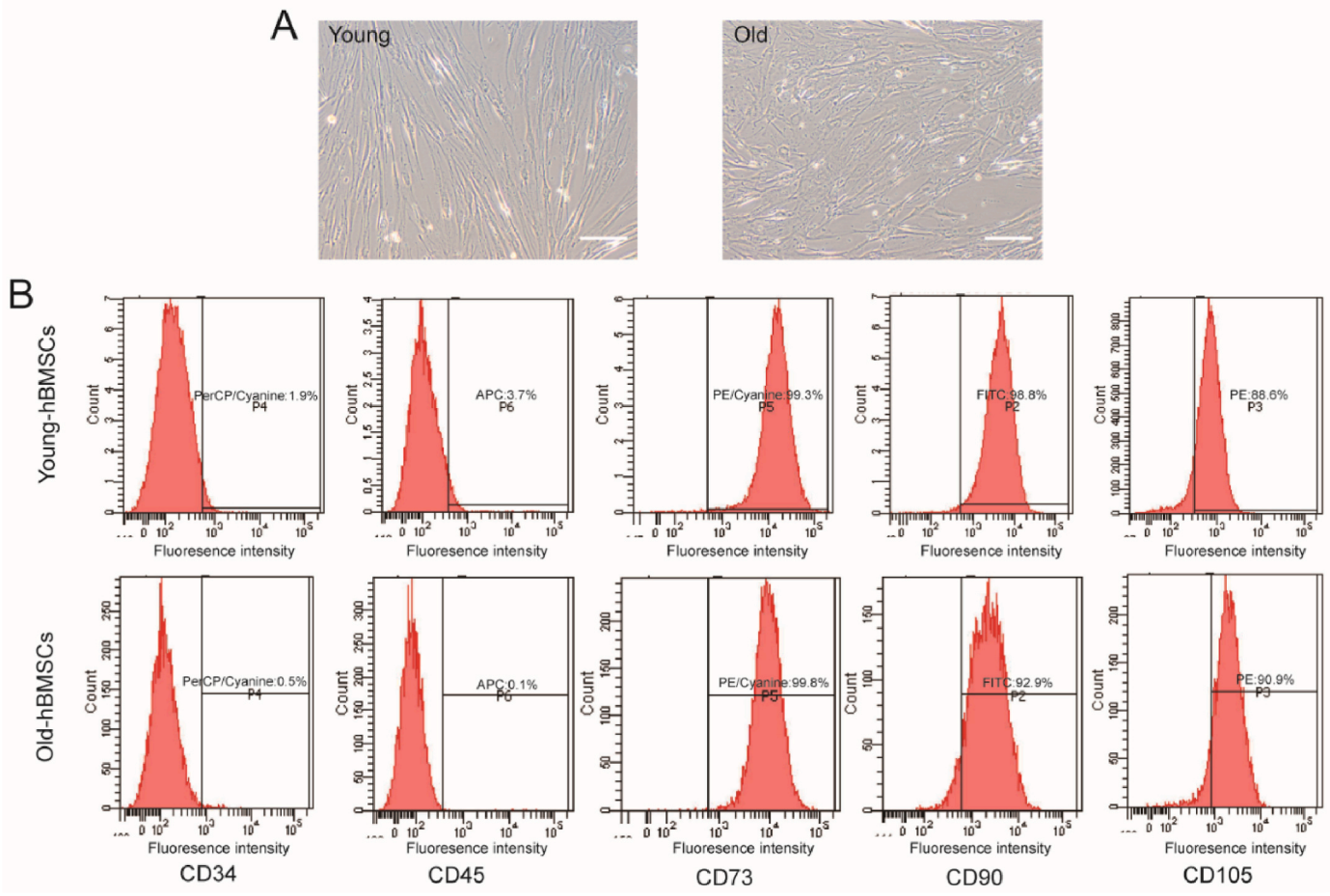

Fig. 1. Characterization and identification of human bone marrow mesenchymal stem cells. (A) Third generation BMSCs, YBMSCs exhibit a spindle and fibroblast morphology and OBMSCs exhibit an elongated flatter fibroblastic morphology (Scale bar, 100 μm). (B) Flow cytometry determination of specific markers in BMSCs showed negative expression of CD34 and CD45 in YBMSCs and OBMSCs, while positive expression of CD73, CD90 and CD105.

Table 2
Hematological variables of each blood component.

Variable	Young (N = 13)	Older (N = 15)	P Value
Age (year)	26 (±3.54)	65.53 (±3.85)	< 0.001
WBC ($\times 10^9$/L)	5.21 (±0.88)	5.28 (±1.23)	0.865
RBC ($\times 10^{12}$/L)	4.40 (±0.18)	4.47 (±0.24)	0.422
PLT ($\times 10^9$/L)	255.23 (±27.15)	247.47 (±19.51)	0.388

Values in the table are shown as the average ± standard deviation.
WBC white blood cells; RBC red blood cells; PLT platelet.

between PRP samples from young and old volunteers. However, IGF-1 (P < 0.001), EGF (P = 0.018), and PDGF-BB (P = 0.001) levels were found to be significantly different, with the younger group having higher levels than the older group, as shown in Table 3.

3.3. Young and old PRP promotes aged BMSCs proliferation

We assessed the effects of PRP on cell proliferation by measuring cell counts, mRNA expression, and immunofluorescence staining of ki-67. We observed the strongest promotion of BMSC growth with a concentration of 50 $\times$ 10^6/ml of PRP (Fig. 2A). Addition of PRP significantly improved the growth of old BMSCs compared to the NC-Old group. Furthermore, the YPRP-Old group showed better growth than the OPRP-Old group, while the young cells (NC-Young) exhibited the highest vigor, resulting in the highest cell numbers among the four groups. This difference was noticeable from day 3 after culture to the end of observation (Fig. 2B). Additionally, ki-67 mRNA expression was higher in old cells cultured with PRP than in control old cells, and expression was significantly higher in the YPRP-Old group than in the OPRP-Old group. However, expression was lower in the YPRP-Old group than in the NC-Young group (Fig. 2C). Immunofluorescence staining for ki-67 showed that the NC-Old group had the least number of positive cells, while the NC-Young group had the highest number of positive cells. PRP culture significantly improved this outcome, but young PRP showed a more significant improvement than old PRP (Fig. 2D and E). In cloning experiments, we found that PRP promoted the cloning ability of old cells. The NC-Young group had the strongest cloning ability, with the highest number of clones and the largest clonal clusters. In contrast, the NC-Old group had the lowest number of clones and the smallest clonal clusters. However, the cloning ability of the PRP group was significantly improved, with the YPRP-Old group performing better than the OPRP-Old group (Fig. 2F and G).

3.4. PRP promotes rejuvenation of aged BMSCs

To investigate whether PRP can promote the rejuvenation of aged BMSCs, we evaluated the mRNA expression levels of senescence-related genes p16 and p21. Our results showed that p16 and p21 expression levels were the highest in the elderly control group and the lowest in the young control group. Moreover, the expression was significantly lower in the PRP culture group, and it was more reduced in the young PRP group than in the elderly PRP group (Fig. 3A, D). Western blot analysis corroborated these PCR results (Fig. 3B–C, E-F). To assess the effect of PRP on BMSCs, senescence-associated β-galactosidase staining was applied. The NC-Young group showed almost no senescent cells, whereas the NC-Old group had the highest number of senescent cells. In contrast, the PRP group showed fewer senescent cells, and the rejuvenation effect of young PRP was better than that of old PRP, indicating that PRP alleviated the senescence of older BMSCs to some extent (Fig. 3G and H). Additionally, by vascularization assay, we found that the NC-Young group exhibited the strongest vascularization, the PRP group performed significantly better than the NC-Old group, and the YPRP-Old group had stronger vascularization than the OPRP-Old group. This suggested that PRP improved the paracrine function of aged BMSCs (Fig. 3I and J).

Table 3
Age differences in selected cytokines and growth factors.

Biomarker	Young (N = 13)	Older (N = 15)	P Value
	Mean (95% CI)	Mean (95% CI)	
TGF-β1 (ng/ml)	38.02 (34.73–41.31)	34.93 (32.18–37.69)	0.129
IGF-1 (ng/ml)	218.65 (187.02–250.29)	145.89 (132.11–159.68)	< 0.001
FGF (pg/ml)	223.53 (198.79–248.27)	195.98 (176.12–215.84)	0.069
EGF (pg/ml)	1168.75 (1053.68–1283.83)	1007.66 (926.97–1088.35)	0.018
PDGF-BB (pg/ml)	8452.05 (7488.23–9415.88)	6330.74 (5503.97–7157.51)	0.001
VEGF (pg/ml)	411.51 (275.18–547.84)	335.51 (265.29–405.72)	0.274

TGF-β1 transforming growth factors-β1; IGF-1 insulin-like growth factor-1; FGF fibroblast growth factor; EGF epidermal growth factor; PDGF-BB platelet derived growth factor-BB; VEGF vascular endothelial growth factor.

3.5. PRP enhancement of aged BMSCs improves cardiac function after myocardial infarction

We conducted an in vivo study on rats to assess the efficacy of cell transplantation for repairing myocardial infarction. Coronary artery ligation in rat hearts resulted in significant ventricular dysfunction, which was evaluated using Masson trichrome staining four weeks after cell transplantation (Fig. 4A). Computerized morphometric analysis revealed a significant reduction in the infarct area after cell treatment compared to the control group. The infarct area in the PRP-cultured groups was smaller than that in the NC-Old group, and more reduced in the YPRP-Old group than in the OPRP-Old group, but still greater than that in the NC-Young group (Fig. 4B). This result indicates that PRP rejuvenated cells are more effective than older control cells in reversing ventricular remodeling after myocardial infarction, particularly young PRP. Echocardiography was performed at 1 and 4 weeks after myocardial infarction (Fig. 4C). LVEF and LVFS were significantly higher in the cell transplantation group than in the control group, and the improvement was more pronounced in the PRP-cultured older cell group than in the NC-Old group. Furthermore, the improvement was more significant in the YPRP-Old group than in the OPRP-Old group. The normal group served as the preoperative control. These findings suggest that PRP rejuvenated cells are more effective than older control cells in improving cardiac function after MI, particularly young PRP (Fig. 4D and E).

3.6. More survival and neovascularization after transplantation of aged hBMSCs after PRP rejuvenation

The survival of cells and neovascularization in the infarcted area were evaluated using immunofluorescence with mitochondrial and α-SMA staining, respectively. The results showed that by week four, cell survival, arterial vascular formation, and capillary density were higher in the PRP-cultured aged cell group than in the NC-Old control group. Additionally, these factors were higher in the YPRP-Old group than in the OPRP-Old group, but still less than in the NC-Young control group (Fig. 5A–D). These findings suggest that PRP culture of aged cells promoted the viability of older cells, and that the rejuvenation effect was better with young PRP than old PRP. The improved cells had stronger anti-apoptotic and angiogenic abilities, which could significantly enhance blood supply in the MI region.

4. Discussion

So far, various experimental and preclinical studies have demonstrated the beneficial effects of MSC-based therapy for ischemic cardiomyopathy. Moreover, a number of clinical trials have also confirmed the safety and efficacy of autologous MSC transplantation(Heldman et al., 2014; Tompkins et al., 2018). However, for the majority of elderly

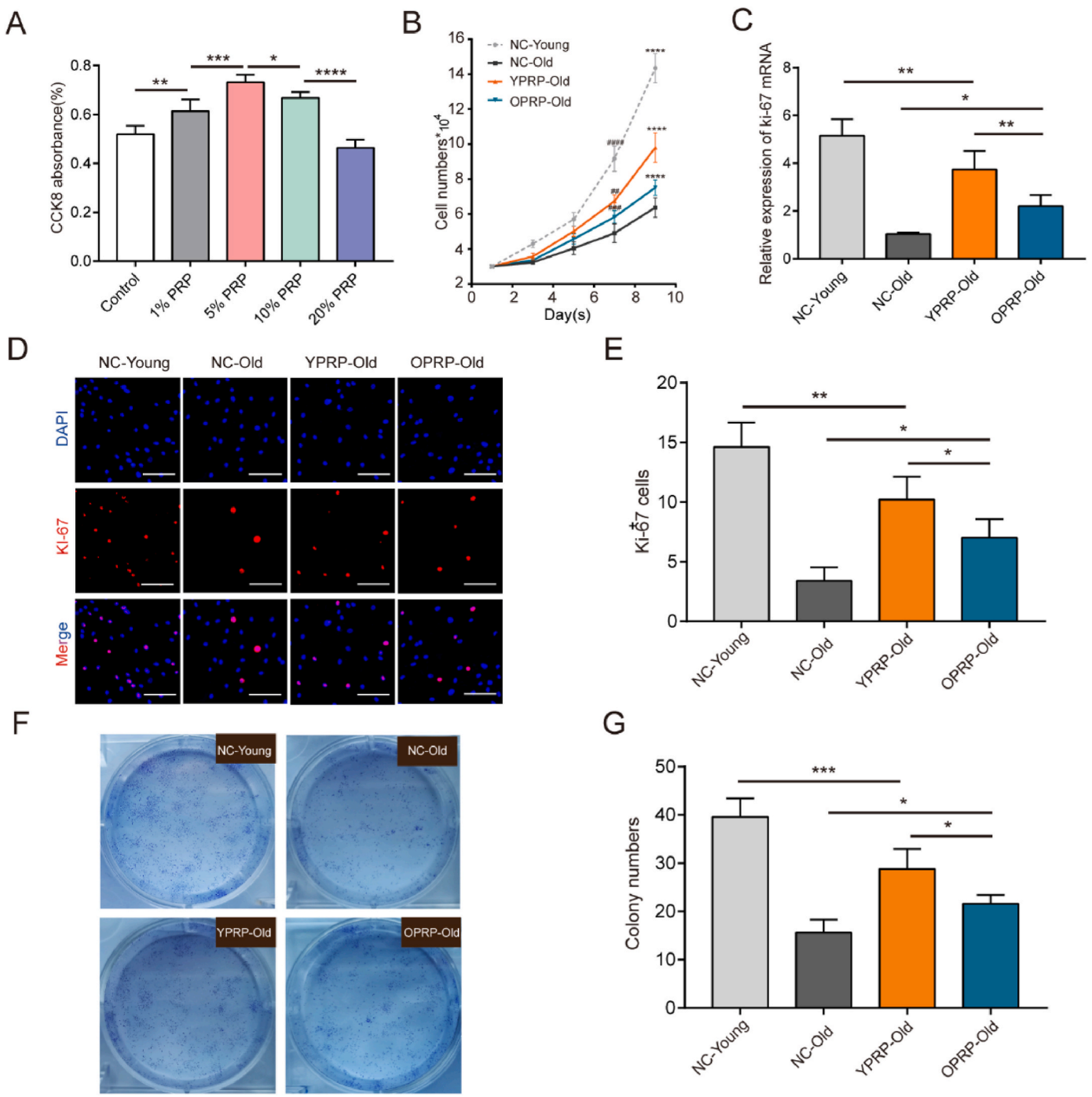

Fig. 2. Effect of young and old PRP on proliferation and clonogenesis of aged BMSCs. (A) CCK8 assay was used to assess the effect of young PRP of 1%, 5%, 10%, and 20% on the proliferation of aged BMSCs. (B) 5% PRP cultured cells were used to assess the growth of BMSCs by cell counting assay at 1, 3, 5, 7 and 9 days. (C) The mRNA expression levels of ki-67 in different groups were measured by qPCR. (D) Proliferation of BMSCs was assessed according to ki-67 immunofluorescence staining (scale bar, 100um). (E) Comparative values of the number of positive cells in each group. (F) Analysis of the effect of PRP on clone formation of aged BMSCs. (G) Quantitative values of clone formation in each group. (Data shown as mean ± SEM; n = 5; ANOVA; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

patients suffering from ischemic heart disease, their own BMSCs are declining in number and their cellular activity is reduced with age, thereby affecting the effectiveness of autologous stem cell transplantation therapy. Our group has previously demonstrated the differences in activity between young and old hBMSCs(Fan et al., 2010). Several rejuvenation strategies have been employed to improve the function of senescent stem cells, such as modulation of relevant intracellular signaling pathways, intervention of epigenetic modifications, and reversal of DNA damage(Zhang et al., 2020; Ren et al., 2017). Nevertheless, these strategies have not been effectively applied in clinical practice due to their limited efficiency and clinical limitations. Therefore, it is of utmost importance to explore a new, simple, effective, and clinically applicable strategy to rejuvenate senescent stem cells.

A model of blood sharing through conjoined symbiosis has been developed to connect the circulatory systems of young and old living animals, allowing their blood to flow together and rejuvenating the phenotype of the older animal(Scudellari, 2015). In this study, we investigated the rejuvenating effect of PRP, which is a "natural growth

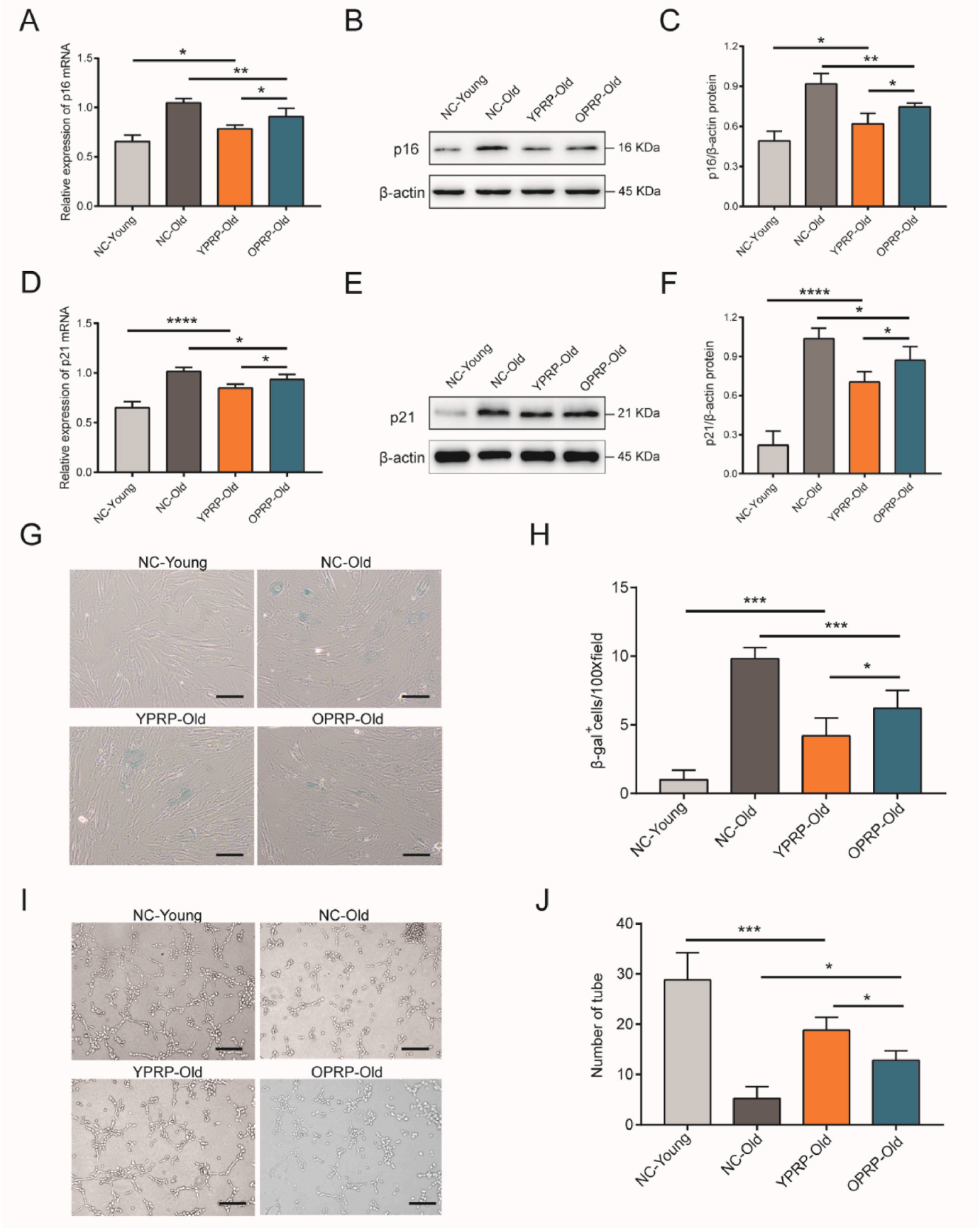

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Fig. 3. PRP promotes aged BMSCs to rejuvenation and angiogenesis. (A) The mRNA expression levels of p16 in the control and experimental groups were determined by qPCR. (B) Protein expression of p16 in the control and experimental groups was detected by Western blot. (C) The relative expression of p16 protein in each group was measured. (D) The mRNA expression levels of p21 in control and experimental groups were determined by qPCR. (E) Protein expression of p21 in the control and experimental groups was detected by Western blot. (F) Measurement of the relative expression of p21 protein in each group. (G) Senescence-associated galactosidase (β-gal) staining showing cellular senescence (scale bar, 100um). (H) Quantitative values of β-gal staining intensity in each group. (I) Analysis of the paracrine effect of PRP to ameliorate senescence in aged cells by angiogenesis assay (scale bar, 100um). (J) Calculation of the number of each constituent vessel. (Data are expressed as mean ± SEM; n = 5; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

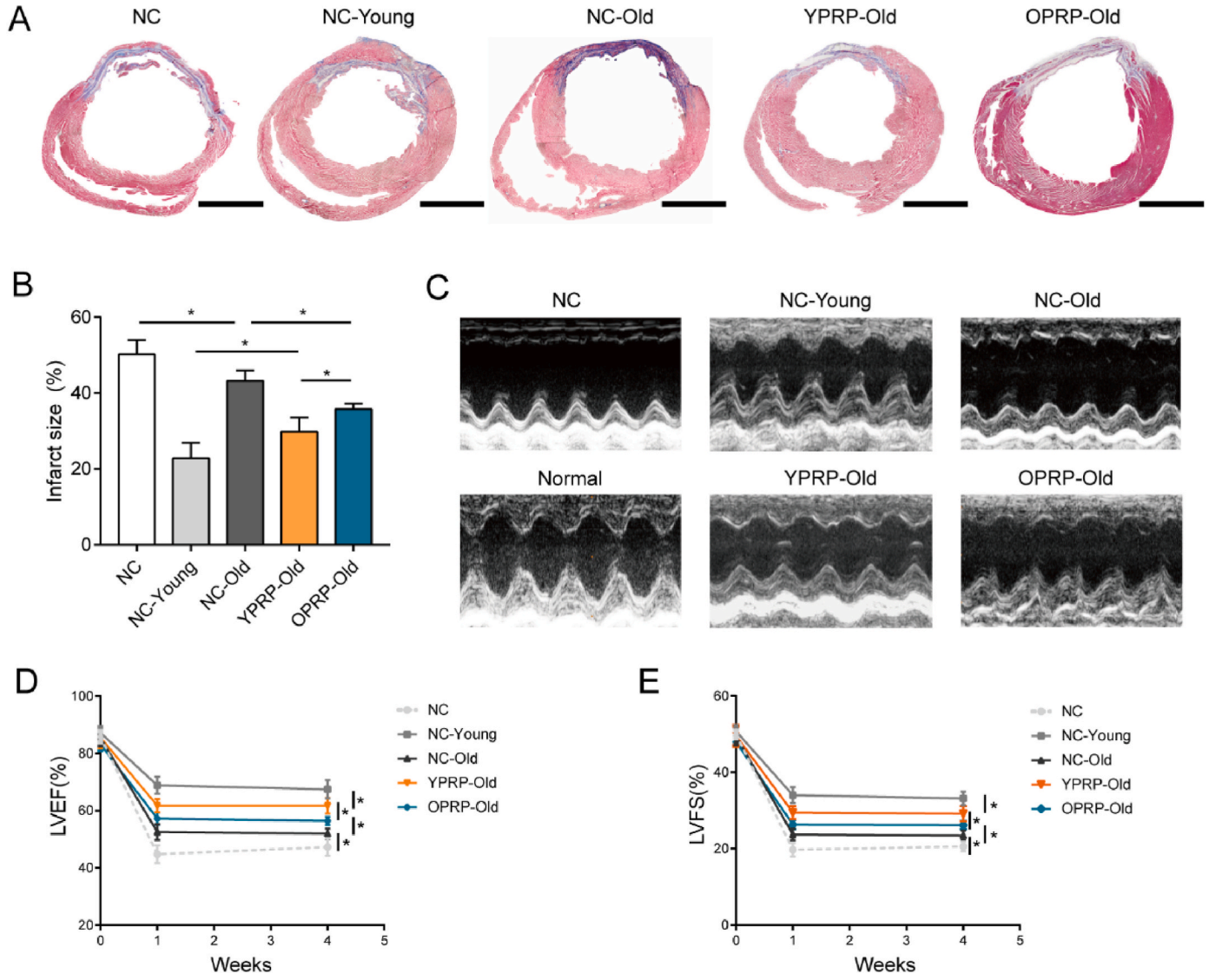

Fig. 4. PRP enhancement of aged BMSCs improves cardiac function after myocardial infarction. (A) Masson trichrome staining of infarct area 4 weeks after transplantation of 5 groups of cells (blue = collagen; red = myocardium), (Scale bar, 2 mm). (B) Comparison of infarct area after 4 weeks in different groups of cells treated for infarction. (C) Representative echocardiographic images of 5 groups of myocardial infarction at 4 weeks (high line: LVESd; low line: LVEDd), normal group is preoperative control. (D–E) Comparison of differences between LVEF and LVFS after cell therapy MI in different subgroups. (Data shown as mean ± SEM; n = 5; ANOVA; *p < 0.05).

factor pool" derived from blood, on aged hBMSCs. We initially compared the main growth factors present in PRP obtained from young and old individuals, and identified significant differences in IGF-1, EGF, and PDGF-BB. It has been demonstrated that IGF-1 and its signaling pathways play a significant role in human growth and aging. IGF-1 is important for neurodevelopment in the human brain, and its reduction with age may contribute to cognitive decline (Frater et al., 2018). Hanwright PJ et al. delivered IGF-1 to damaged nerve sites in rats, which significantly improved the recovery of forepaw grip strength, reduced denervation-induced muscle atrophy, reduced Schwann cell senescence, and improved neuromuscular reinnervation(Hanwright et al., 2022). Thus, it is hypothesized that the higher levels of IGF-1 in PRP from young individuals are a crucial factor in enabling enhanced function and rejuvenation of older hBMSCs. EGF has been reported to play an important role in cell proliferation, differentiation, and growth, and it is capable of repairing and restoring the function of hematopoietic stem cells damaged by radiotherapy(Fang et al., 2020). Therefore, EGF plays an important role in repairing aged hBMSCs and reversing damage. Furthermore, PDGF has been shown to play a significant role in inducing stem cell proliferation and angiogenesis(Fortier et al., 2011; Gharibi and

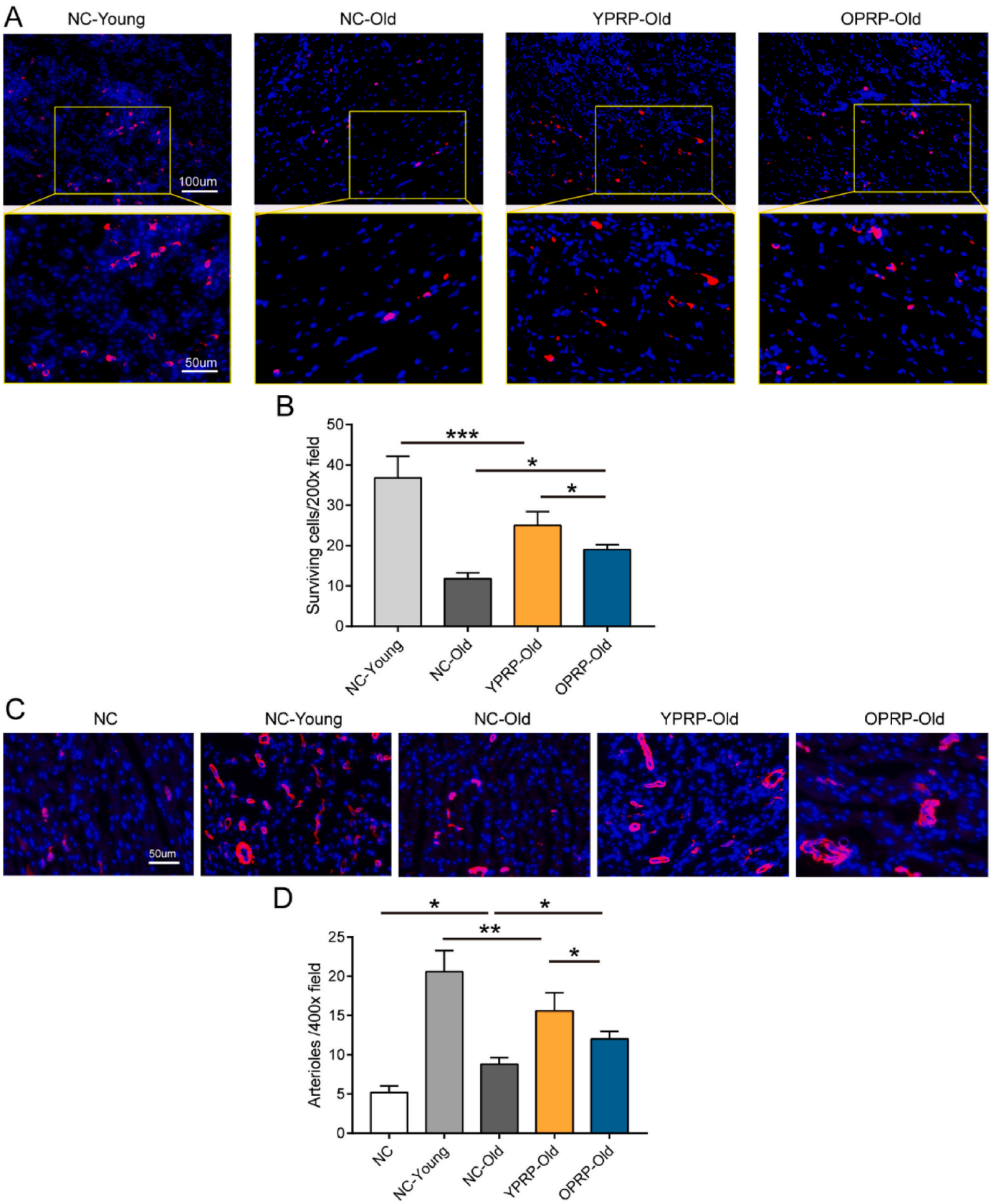

Fig. 5. More survival and neovascularization after transplantation of aged hBMSCs after PRP rejuvenation. (A) Anti-human mitochondrial staining showing the survival rate of transplanted cells (scale bar, 100um vs. 50um). (B) Comparative values of the number of surviving cells in the experimental group versus the control group 28 days after cell transplantation. (C) Staining for α-SMA was used to observe vascular density. (D) Comparative values of the number of capillaries in the experimental group versus the control group at 28 days after MI. (Data are shown as mean ± SEM; n = 5; ANOVA; *p < 0.05, **p < 0.01, ***p < 0.001).

Hughes, 2012). These growth factors are crucial for enhancing the function of hBMSCs. Additionally, there may be other unknown rejuvenation factors present in young blood that also contribute to rejuvenation, which is a direction we plan to investigate in future studies. TGF-β1, FGF, and VEGF did not show any significant differences, which may be due to the small sample size.

Next, we evaluated the effect of PRP on cell proliferation. Although the pro-proliferation properties of PRP have been reported in infection and tissue engineering, its effect on aged hBMSCs has not been extensively studied (Zhang et al., 2019; Santos et al., 2018). However, there have been limited studies on the effect of PRP from young and old people on aged hBMSCs. In our study, we found that the growth rate of aged cells cultured with PRP was significantly better than that of aged control cells, and the YPRP-Old group grew significantly better than the OPRP-Old group. These findings were also confirmed by ki-67 gene expression through immunofluorescence staining. Furthermore, young PRP demonstrated better improvement in clonogenesis compared to old PRP. These results indicate that PRP-optimized aged hBMSCs derived from humans can increase cell activity by promoting growth and clonogenesis, and young PRP is more effective than aged PRP. PRP has been used in various clinical applications in recent years(Hong et al., 2022; Karjalainen et al., 2021), and our study provides further evidence for the practical application of young PRP.

We then focused on investigating the rejuvenating effects of human PRP on aged hBMSCs, with a particular emphasis on two key biomarkers of cellular senescence, namely, p16 and p21, which are both dependent on cell cycle protein kinases (Sturmlechner et al., 2017; Baker et al., 2016). Firstly, we evaluated the mRNA expression levels of the senescence-associated genes p16 and p21. We found that culturing aged hBMSCs with PRP led to a significant reduction in p16 and p21 gene expression compared to control aged cells. This result was further confirmed at the protein level by Western blot analyses of p16 and p21. Interestingly, the PRP obtained from young adults demonstrated a more potent effect in this regard. Furthermore, we observed that PRP derived from young adults significantly reduced the number of hBMSCs stained by aging-related β-galactosidase staining, albeit to a lesser extent with PRP from old adults. These findings suggest that human-derived PRP plays a role in rejuvenating aged hBMSCs to some extent.Stem cells exert their reparative effects on tissues mainly through differentiation into tissue-specific cells and paracrine signaling. As cells age, their paracrine function also declines, which may contribute to the accumulation of more senescent cells and further dysregulation of surrounding cells through the spread of inflammatory factors to neighboring cells (He and Sharpless, 2017). Therefore, we investigated the effect of PRP on the paracrine secretion of aged BMSCs using an angiogenesis assay. We found that PRP promoted the angiogenesis of aged BMSCs, with a stronger effect observed with young PRP. These results suggest that the paracrine function of aged BMSCs may be partially improved by the effects of human PRP.

Finally, we evaluated the therapeutic effect in an SD rat infarction model, and the group treated with PRP-optimized aged hBMSCs showed a more significant improvement in cardiac function, reduced MI area, and increased vessel formation to improve blood supply to the infarcted area compared to the aged control BMSC group. This may be attributed to the stronger anti-apoptotic and angiogenic abilities of the PRP-optimized cells. Additionally, PRP plays a crucial role in enhancing the paracrine function of transplanted cells. Moreover, as both BMSCs and PRP are of human origin and almost non-immunogenic, this method may become a simple, targeted, efficient, and safe strategy for clinical application in the future. However, the underlying mechanism of rejuvenation of older BMSCs by PRP needs to be further investigated.

The main finding of this study is that PRP obtained from both younger and older donors exhibited proliferative and rejuvenating effects on aged BMSCs, with PRP from younger donors showing superior efficacy. As a result, aged BMSCs optimized with young PRP may be more suitable for autologous cell transplantation. This study is the first to demonstrate that human PRP can rejuvenate aged BMSCs, offering a novel pathway for the rejuvenation of senescent BMSCs in elderly patients and representing a promising therapeutic intervention for the treatment of ischemic heart disease and other aging-related conditions.

5. Conclusion

We demonstrate, for the first time, that human PRP can rejuvenate aged hBMSCs and that PRP derived from younger adults is superior in this regard. In our MI rat model, the YPRP-Old group outperformed the OPRP-Old group, suggesting that young PRP has significant translational potential and could be a promising clinical approach for improving cell therapy in the treatment of IHD.

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CRediT authorship contribution statement

Erliang Guo: conducted the study, processed the data and performed the statistical analysis, wrote the manuscript. **Lu Sun:** conducted the study, conceived the study together and drafted the manuscript. **Wei Chen:** conducted the study, processed the data and performed the statistical analysis. **Chang Liu:** conducted the study. **Kegong Chen:** conducted the study. **Xingpei Jiang:** conducted the study. **Xionghai Qin:** conducted the study. **Jianling Su:** conducted the study. **Fan Yang:** conducted the study. **Hai Tian:** wrote the manuscript, conceived, the study together and drafted the manuscript. all authors reviewed and approved the final version of the manuscript.

Declaration of competing interest

All authors claim that there are no conflicts of interest.

Data availability

Data will be made available on request.

References

Arnett, D.K., Blumenthal, R.S., Albert, M.A., Buroker, A.B., Goldberger, Z.D., Hahn, E.J., Himmelfarb, C.D., Khera, A., Lloyd-Jones, D., McEvoy, J.W., Michos, E.D., Miedema, M.D., Munoz, D., Smith Jr., S.C., Virani, S.S., Williams, K.A., Sr ., Yeboah, J., Ziaeian, B., 2019. '2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: a report of the American college of cardiology/American heart association task force on clinical practice guidelines. J. Am. Coll. Cardiol. 74, e177–e232.

Bai, L., Sun, L., Chen, W., Liu, K.Y., Zhang, C.F., Wang, F., Zhang, G.H., Huang, Y., Li, J. X., Gao, Y., Sun, X., Liu, W., Du, G.Q., Li, R.K., Huang, M.L., Tian, H., 2020. 'Evidence for the existence of CD34(+) angiogenic stem cells in human first-trimester decidua and their therapeutic for ischaemic heart disease. J. Cell Mol. Med. 24, 11837–11848.

Baker, D.J., Childs, B.G., Durik, M., Wijers, M.E., Sieben, C.J., Zhong, J., Saltness, R.A., Jeganathan, K.B., Verzosa, G.C., Pezeshki, A., Khazaie, K., Miller, J.D., van Deursen, J.M., 2016. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Nature 530, 184–189.

Dimmeler, S., Leri, A., 2008. Aging and disease as modifiers of efficacy of cell therapy. Circ. Res. 102, 1319–1330.

Fan, M., Chen, W., Liu, W., Du, G.Q., Jiang, S.L., Tian, W.C., Sun, L., Li, R.K., Tian, H., 2010. The effect of age on the efficacy of human mesenchymal stem cell transplantation after a myocardial infarction. Rejuvenation Res. 13, 429–438.

Fan, M., Huang, Y., Chen, Z., Xia, Y., Chen, A., Lu, D., Wu, Y., Zhang, N., Qian, J., 2019. 'Efficacy of mesenchymal stem cell therapy in systolic heart failure: a systematic review and meta-analysis. Stem Cell Res. Ther. 10, 150.

Fang, T., Zhang, Y., Chang, V.Y., Roos, M., Termini, C.M., Signaevskaia, L., Quarmyne, M., Lin, P.K., Pang, A., Kan, J., Yan, X., Javier, A., Pohl, K., Zhao, L., Scott, P., Himburg, H.A., Chute, J.P., 2020. Epidermal growth factor receptor-

dependent DNA repair promotes murine and human hematopoietic regeneration. Blood 136, 441–454.
Fortier, L.A., Barker, J.U., Strauss, E.J., McCarrel, T.M., Cole, B.J., 2011. The role of growth factors in cartilage repair. Clin. Orthop. Relat. Res. 469, 2706–2715.
Frater, J., Lie, D., Bartlett, P., McGrath, J.J., 2018. 'Insulin-like Growth Factor 1 (IGF-1) as a marker of cognitive decline in normal ageing: a review. Ageing Res. Rev. 42, 14–27.
Gharibi, B., Hughes, F.J., 2012. 'Effects of medium supplements on proliferation, differentiation potential, and in vitro expansion of mesenchymal stem cells. Stem Cells Transl Med 1, 771–782.
Gong, R., Jiang, Z., Zagidullin, N., Liu, T., Cai, B., 2021. Regulation of cardiomyocyte fate plasticity: a key strategy for cardiac regeneration. Signal Transduct. Targeted Ther. 6, 31.
Goradel, N.H., Hour, F.G., Negahdari, B., Malekshahi, Z.V., Hashemzehi, M., Masoudifar, A., Mirzaei, H., 2018. 'Stem cell therapy: a new therapeutic option for cardiovascular diseases. J. Cell. Biochem. 119, 95–104.
Hanwright, P.J., Qiu, C., Rath, J., Zhou, Y., von Guionneau, N., Sarhane, K.A., Harris, T. G.W., Howard, G.P., Malapati, H., Lan, M.J., Reddy, S., Hoke, A., Mao, H.Q., Tuffaha, S.H., 2022. 'Sustained IGF-1 delivery ameliorates effects of chronic denervation and improves functional recovery after peripheral nerve injury and repair. Biomaterials 280, 121244.
Harrell, C.R., Fellabaum, C., Jovicic, N., Djonov, V., Arsenijevic, N., Volarevic, V., 2019. Molecular Mechanisms Responsible for Therapeutic Potential of Mesenchymal Stem, vol. 8. Cell-Derived Secretome, Cells.
He, S., Sharpless, N.E., 2017. Senescence in health and disease. Cell 169, 1000–1011.
Heldman, A.W., DiFede, D.L., Fishman, J.E., Zambrano, J.P., Trachtenberg, B.H., Karantalis, V., Mushtaq, M., Williams, A.R., Suncion, V.Y., McNiece, I.K., Ghersin, E., Soto, V., Lopera, G., Miki, R., Willens, H., Hendel, R., Mitrani, R., Pattany, P., Feigenbaum, G., Oskouei, B., Byrnes, J., Lowery, M.H., Sierra, J., Pujol, M.V., Delgado, C., Gonzalez, P.J., Rodriguez, J.E., Bagno, L.L., Rouy, D., Altman, P., Foo, C.W., da Silva, J., Anderson, E., Schwarz, R., Mendizabal, A., Hare, J.M., 2014. 'Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: the TAC-HFT randomized trial. JAMA 311, 62–73.
Hesseler, M.J., Shyam, N., 2019. 'Platelet-rich plasma and its utility in medical dermatology: a systematic review. J. Am. Acad. Dermatol. 81, 834–846.
Hong, P., Zheng, Y., Rai, S., Ding, Y., Zhou, Y., Liu, R., Li, J., 2022. 'Efficacy and safety of platelet-rich plasma in the treatment of carpal tunnel syndrome: a network meta-analysis of different injection treatments. Front. Pharmacol. 13, 906075.
Karjalainen, T.V., Silagy, M., O'Bryan, E., Johnston, R.V., Cyril, S., Buchbinder, R., 2021. 'Autologous blood and platelet-rich plasma injection therapy for lateral elbow pain. Cochrane Database Syst. Rev. 9, CD010951.
Kong, Y., Ma, B., Liu, F., Chen, D., Zhang, S., Duan, J., Huang, Y., Sang, Y., Wang, J., Li, D., Liu, H., Wang, S., 2019. 'Cellular stemness maintenance of human adipose-derived stem cells on ZnO nanorod arrays. Small 15, e1904099.
Li, M., Zhang, T., Jiang, J., Mao, Y., Zhang, A., Zhao, J., 2019. 'ECM coating modification generated by optimized decellularization process improves functional behavior of BMSCs. Mater Sci Eng C Mater Biol Appl 105, 110039.
Liu, H.Y., Huang, C.F., Lin, T.C., Tsai, C.Y., Tina Chen, S.Y., Liu, A., Chen, W.H., Wei, H. J., Wang, M.F., Williams, D.F., Deng, W.P., 2014. Delayed animal aging through the recovery of stem cell senescence by platelet rich plasma. Biomaterials 35, 9767–9776.
Mao, G., Zhang, G., Fan, W., 2019. 'Platelet-Rich plasma for treating androgenic alopecia: a systematic review. Aesthetic Plast. Surg. 43, 1326–1336.
Mehranfar, S., Abdi Rad, I., Mostafav, E., Akbarzadeh, A., 2019. 'The use of stromal vascular fraction (SVF), platelet-rich plasma (PRP) and stem cells in the treatment of osteoarthritis: an overview of clinical trials. Artif. Cells, Nanomed. Biotechnol. 47, 882–890.
Ren, R., Ocampo, A., Liu, G.H., Izpisua Belmonte, J.C., 2017. Regulation of stem cell aging by metabolism and epigenetics. Cell Metabol. 26, 460–474.
Roth, G.A., Mensah, G.A., Johnson, C.O., Addolorato, G., Ammirati, E., Baddour, L.M., Barengo, N.C., Beaton, A.Z., Benjamin, E.J., Benziger, C.P., Bonny, A., Brauer, M., Brodmann, M., Cahill, T.J., Carapetis, J., Catapano, A.L., Chugh, S.S., Cooper, L.T., Coresh, J., Criqui, M., DeCleene, N., Eagle, K.A., Emmons-Bell, S., Feigin, V.L., Fernandez-Sola, J., Fowkes, G., Gakidou, E., Grundy, S.M., He, F.J., Howard, G., Hu, F., Inker, L., Karthikeyan, G., Kassebaum, N., Koroshetz, W., Lavie, C., Lloyd-Jones, D., Lu, H.S., Mirijello, A., Temesgen, A.M., Mokdad, A., Moran, A.E., Muntner, P., Narula, J., Neal, B., Ntsekhe, M., Moraes de Oliveira, G., Otto, C., Owolabi, M., Pratt, M., Rajagopalan, S., Reitsma, M., Ribeiro, A.L.P., Rigotti, N., Rodgers, A., Sable, C., Shakil, S., Sliwa-Hahnle, K., Stark, B., Sundstrom, J., Timpel, P., Tleyjeh, I.M., Valgimigli, M., Vos, T., Whelton, P.K., Yacoub, M., Zuhlke, L., Murray, C., Fuster, V., Gbd-Nhlbi-Jacc Global Burden of Cardiovascular Diseases Writing Group, 2020. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study. J. Am. Coll. Cardiol. 76, 2982–3021.
Santos, Scnds, Sigurjonsson, O.E., Custodio, C.A., Mano, Jfcdl, 2018. 'Blood plasma derivatives for tissue engineering and regenerative medicine therapies. Tissue Eng. B Rev. 24, 454–462.
Scudellari, M., 2015. 'Ageing research: blood to blood. Nature 517, 426–429.
Sturmlechner, I., Durik, M., Sieben, C.J., Baker, D.J., van Deursen, J.M., 2017. 'Cellular senescence in renal ageing and disease. Nat. Rev. Nephrol. 13, 77–89.
Tompkins, B.A., Rieger, A.C., Florea, V., Banerjee, M.N., Natsumeda, M., Nigh, E.D., Landin, A.M., Rodriguez, G.M., Hatzistergos, K.E., Schulman, I.H., Hare, J.M., 2018. 'Comparison of mesenchymal stem cell efficacy in ischemic versus nonischemic dilated cardiomyopathy. J. Am. Heart Assoc. 7.
Xu, J., Gou, L., Zhang, P., Li, H., Qiu, S., 2020. 'Platelet-rich plasma and regenerative dentistry. Aust. Dent. J. 65, 131–142.
Yu, H., Lu, K., Zhu, J., Wang, J., 2017. 'Stem cell therapy for ischemic heart diseases. Br. Med. Bull. 121, 135–154.
Zhang, D.Y., Gao, T., Xu, R.J., Sun, L., Zhang, C.F., Bai, L., Chen, W., Liu, K.Y., Zhou, Y., Jiao, X., Zhang, G.H., Guo, R.L., Li, J.X., Gao, Y., Jiao, W.J., Tian, H., 2020. 'SIRT3 transfection of aged human bone marrow-derived mesenchymal stem cells improves cell therapy-mediated myocardial repair. Rejuvenation Res. 23, 453–464.
Zhang, D.Y., Zhang, C.F., Fu, B.C., Sun, L., Wang, X.Q., Chen, W., Liu, W., Liu, K.Y., Du, G.Q., Ma, C.Y., Jiang, S.L., Li, R.K., Tian, H., 2018a. 'Sirtuin3 protects aged human mesenchymal stem cells against oxidative stress and enhances efficacy of cell therapy for ischaemic heart diseases. J. Cell Mol. Med. 22, 5504–5517.
Zhang, W., Guo, Y., Kuss, M., Shi, W., Aldrich, A.L., Untrauer, J., Kielian, T., Duan, B., 2019. 'Platelet-Rich plasma for the treatment of tissue infection: preparation and clinical evaluation. Tissue Eng. B Rev. 25, 225–236.
Zhang, W., Song, M., Qu, J., Liu, G.H., 2018b. Epigenetic modifications in cardiovascular aging and diseases. Circ. Res. 123, 773–786.