Research Article

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Regenerative Medicine

Hyperacute serum has markedly better regenerative efficacy than platelet-rich plasma in a human bone oxygen–glucose deprivation model

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Aim: Platelet-rich plasma (PRP) and hyperacute serum (HAS) were compared in a novel human model of *ex vivo* bone damage induced by oxygen–glucose deprivation (OGD). **Materials & methods:** Osteoarthritic subchondral bone pieces were harvested from discarded femoral heads during hip replacement surgery and subjected to transient OGD. **Results:** Proteome profiling revealed that PRP is more angiopoietic, whereas HAS is more antiangiopoietic in composition. However, treatment of OGD-exposed bone with multiple PRP preparations had no effect on cell counts, whereas HAS restored cell proliferation capacity and rescued viable cell number following OGD. **Conclusion:** A similar pro-proliferation effect was observed with recombinant growth factors, indicating that HAS may be an alternative agent for enhancing the regeneration of damaged bone cells.

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Keywords: blood fractions • bone necrosis • femoral head • growth factors • ischemia

Recent advances in regenerative medicine have shed light on the capabilities of growth factors such as recombinant bone morphogenic proteins (BMP)-2 and -7 as inducers of bone formation [1–3]. In addition to individual growth factors, several blood serum fractions containing multiple bioactive proteins have shown osteogenic potential [4,5]. Out of these, platelet releasate from platelet-rich plasma (PRP) is the most widely applied plasma-derived blood separation product, and recent double-blind controlled studies provided strong evidence that PRP injections can significantly improve the symptoms of early-stage knee osteoarthritis (OA) [6–10]. There are, however, significant drawbacks that have limited the broader acceptance and clinical use of PRP, chief among them being the high constituent variability among PRP preparations and unclear mechanisms of action [11–13]. The current recommended PRP-based protocol for OA treatment involves injection of PRP releasate produced by $CaCl_2$ or thrombin activation [14,15]; however, this is by no means a consensus approach. Therefore, there is a need for better understanding of the tissue-level actions of such biological treatments.

Given these difficulties and inconsistencies associated with PRP preparation and use, preclinical and clinical investigations have begun to focus on alternate compounds such as platelet-rich fibrin (PRF), a serum fraction containing a multitude of growth factors also found in PRP [16,17] but with potentially more predictable composition and modes of action. Platelet-rich fibrin has been shown to promote bone growth and regeneration both *in vitro* and *in vivo* [18,19]. Much of this early work was conducted on models of oral maxillofacial repair, which successfully

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demonstrated the ability of PRF to promote and augment new bone growth [20–22]. Thus, PRF has the potential to promote osteogenesis and healing of orthopedic conditions.

Platelet-rich fibrin and PRP belong to a relatively new class of therapeutic agents that are not well studied outside of transfusion medicine. In contrast to small molecule drugs, these serum fractions are applied mainly in an autologous manner to avoid the remote chance of viral transmission [23,24]. Expectations towards their composition, target profile, efficacy and safety are thus very different, making autologous biologic compounds incompatible with categories designed for defined chemical entities. Indeed, PRP is not merely a vehicle for delivery of yet unknown platelet-derived factors, but rather a biological mixture that can target multiple body systems [25,26]. As such, the *in vitro* assays used to investigate targeted effects on individual cellular processes, such as membrane transport studies for the discovery of channel inhibitors, are less relevant for serum products with potentially heterogeneous targets such as PRP. Thus, it is of the utmost importance to investigate the effects of PRP and analogous compounds in models that mimic the relevant clinical condition.

In an earlier study, we reported that hyperacute serum (HAS), a cell- and fibrin-free blood derivative extracted from PRF, promotes proliferation of human bone marrow-derived mesenchymal stem cells and influences adipogenic differentiation [27]. In a subsequent experiment we compared the effect of PRP and HAS on chondrocyte proliferation in monolayers. We found that HAS affects chondrocyte proliferation, while PRP enhances both proliferation and redifferentiation of dedifferentiated chondrocytes [28]. In the present study, we tested the regenerative potential of HAS in a novel *ex vivo* human model of bone ischemia that closely recapitulates the pathology of end-stage degenerative bone disease. Subchondral bone fragments extracted from human femoral heads were placed in optimized cell culture conditions and subjected to controlled oxygen–glucose deprivation (OGD), followed by assessment of bone cell viability. We hypothesized that both PRP and HAS would act to protect osteocytes or stimulate subsequent regeneration.

Materials & methods

Isolation & activation of PRP

PRP was isolated by the classical double-centrifugation protocol [29]. Venous blood samples (6 ml) from 20 healthy adult donors were collected in anticoagulant tubes (BD Vacutainer®, K_2E EDTA) in compliance with general blood donation requirements and centrifuged at 320 x *g* for 12 min at RT. The supernatant plasma, including the platelet-rich buffy coat, was completely removed and centrifuged at 1710 *g* for 10 min, after which 2/3 of the supernatant, referred to as platelet-poor plasma (PPP), was removed and the pellet resuspended in the remaining supernatant. The resultant preparation was used as native PRP. Portions of the native PRP were then post-treated in various ways for *ex vivo* experiments. Native PRP was activated by adding $CaCl_2$ and/or thrombin, treated with heparin to prevent formation of coagula in the cell culture medium during experiments, or subjected to repeated freezing and thawing to produce cell-free releasate (Figure 1).

Isolation of HAS

PRF was prepared by centrifugation of 6 ml whole blood samples without anticoagulants for 5–10 min at 1710 x *g* at RT, during which a fibrinous gel was formed in the supernatant [30]. This PRF gel was then removed from the tube and the fluid gently squeezed out, yielding HAS (Figure 1).

Bone explants

40 bone explants were harvested from each discarded femoral head of patients undergoing hip replacement surgery. Bone grafts of about 10 mm^3 were collected and transferred immediately into stem cell medium (Dulbecco's Modified Eagle Medium containing 1 g/l glucose, 1% penicillin–streptomycin and 10% fetal bovine serum) and maintained at 37°C under a humidified atmosphere of 5% CO_2 for 3 days prior to treatment [31]. In total, 855 bone explants were used in this study: 144 to determine the optimal OGD duration (7 h) and provide control data; 298 for examining the effects of the various PRP preparations; 244 for assessing the effects of HAS; and 169 for studies on recombinant growth factors.

Oxygen–glucose deprivation

Oxygen–glucose deprivation was performed in a Pecon incubation system (Erbach-Bach, Germany) on the third day after explant preparation (Figure 2). The cultured bone pieces were transferred into glucose- and amino acid-free

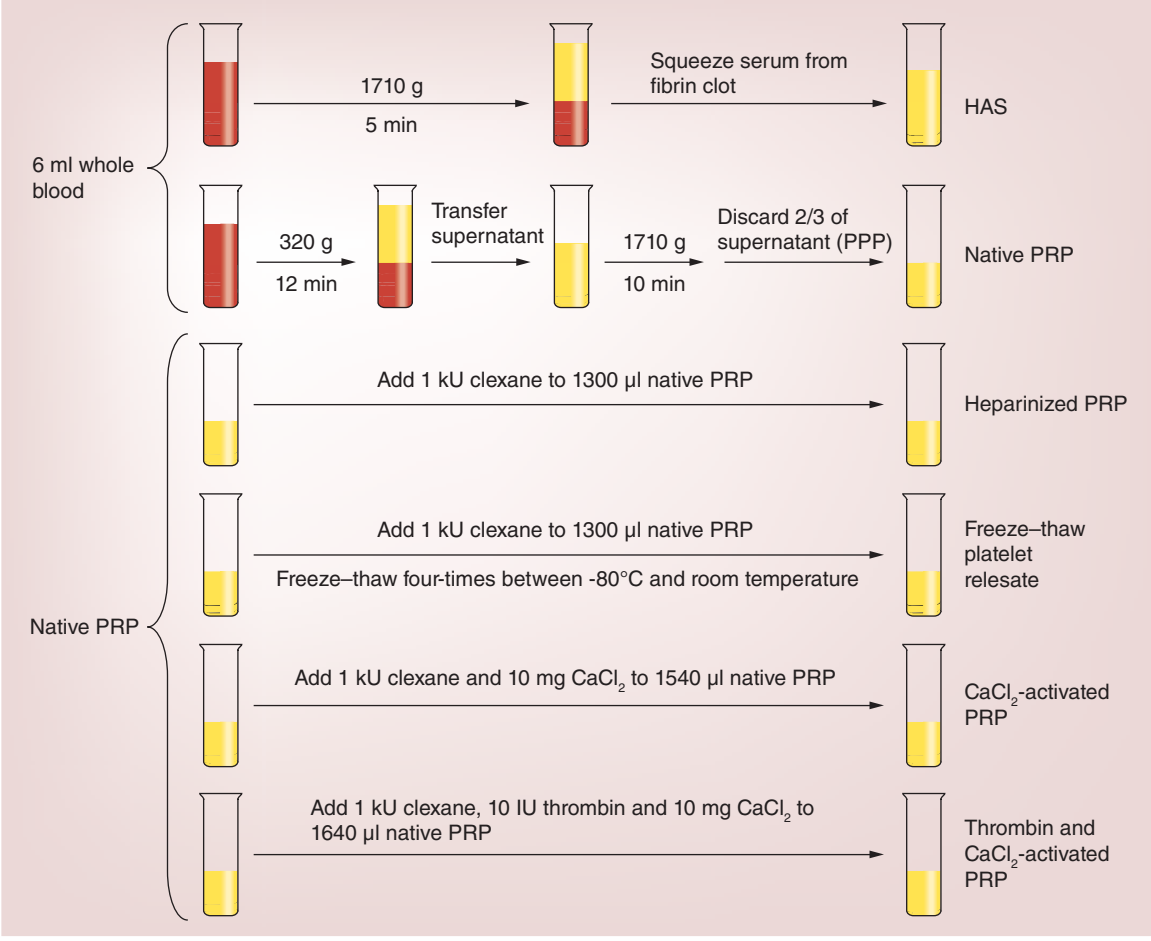

Figure 1. Schematic overview of native platelet-rich plasma and hyperacute serum preparation.
Hyperacute serum was prepared in two steps: centrifugation of serum with anticoagulants to form a fibrinous gel supernatant, and squeezing out the liquid from the fibrin matrix. PRP was prepared in four steps: centrifugation in an anticoagulant-containing tube, recentrifugation of the first supernatant, removal of the PPP, (the top 2/3 of the supernatant) and resuspension of the pellet in the remaining supernatant yielded native PRP. The native PRP was used directly or alternatively heparinized, disrupted by freeze–thawing to produce releasate, or activated by thrombin and/or $CaCl_2$.
HAS: Hyperacute serum; PPP: Platelet-poor plasma; PRP: Platelet-rich plasma.

medium, and the oxygen was flushed out with nitrogen for 7 h. The cultures were then reperfused and maintained under normal cell culture conditions with periodic assessment of cell viability for at least 6 days.

Treatment of explants with blood fractions & recombinant growth factors

Blood fractions were added to the medium at a ratio of 1:4 before or immediately after OGD as indicated and refreshed during medium changes. Both PRP and HAS were prepared fresh immediately before use. Recombinant PF-4 was applied in a similar manner as HAS or PRP in doses of 10, 100 and 1000 ng/ml, whereas BMP-2, BMP-7 and PDGF were added in doses of 5, 50 and 500 ng/ml.

Cell viability was quantified by the MTT assay. Briefly, explants treated as indicated were incubated in a 1:9 diluted mixture of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, #M5655, Sigma, MO, USA) and stem cell medium at 37°C for 60 min. The formazan crystals formed by viable cells were then dissolved in isopropanol, and the absorbance of the solution, which is proportional to viable cell number, was measured using a Biotek PowerWave-XS spectrophotometer at 570 nm. Formazan absorbance measures were background corrected by measuring the absorbance at 690 nm. The MTT assay was performed on the third and sixth days after OGD (days 6 and 9 *ex vivo*, respectively). Cell viability results were verified by staining the cells

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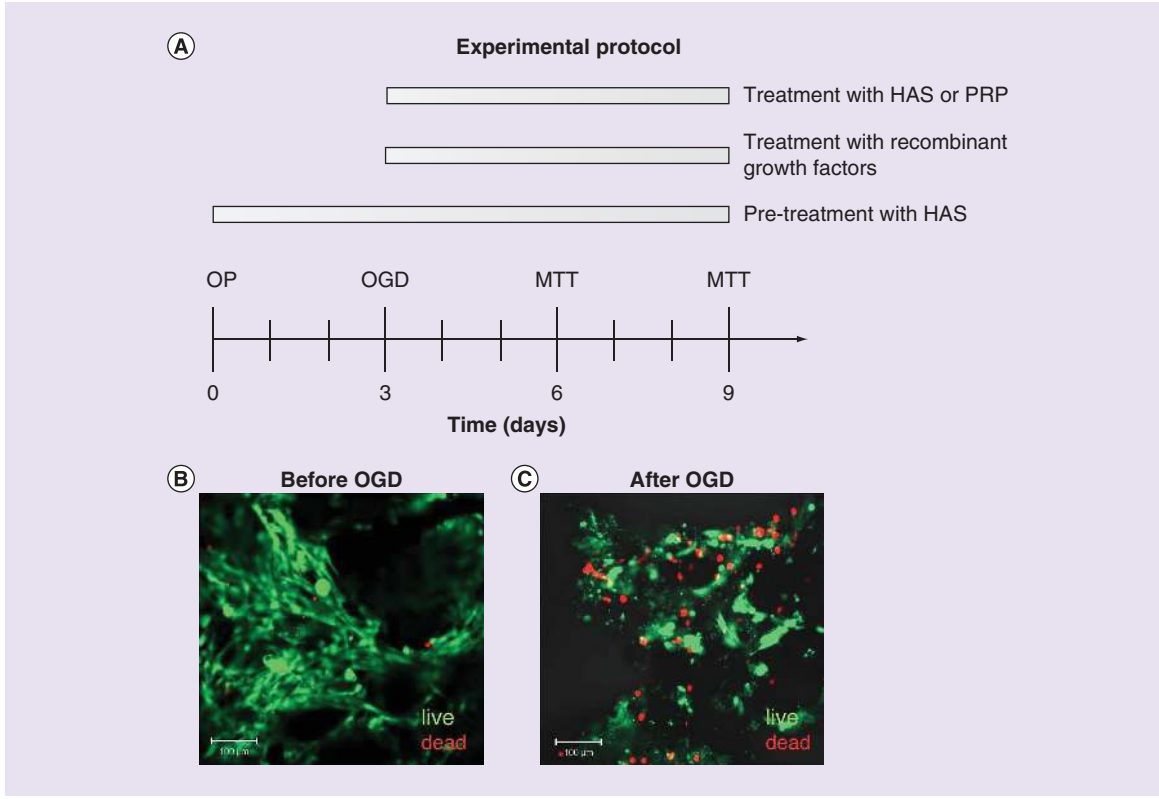

Figure 2. Experimental model. (A) Timeline of human bone explant culture, simulated ischemia (OGD) and post-OGD examination of cell viability. Bone tissue pieces were harvested from femoral heads (day 0) and kept in culture for 3 days. **(B)** Such preparations showed good cell viability as demonstrated by fluorescent live/dead cell assay. **(C)** Ischemia was simulated by OGD for 7 h on day 3, followed by replacement of normal stem cell medium and a return to normoxia (20%). At 3 days after OGD, viable cell number was reduced by approximately 80%. Serum fractions were added to the explanted cultures just before or after OGD as indicated and replaced at medium changes when necessary until the end of the experiment. Cell viability was measured on either the sixth or the ninth day *ex vivo* (third or sixth day post-OGD) by replacing the tissues in a fresh well and thus measuring the cells on the bone matrix only. Qualitative assessment of cell viability was performed by labelling with calcein-AM (488 nm) and ethidium homodimer-2 (546 nm) and evaluated by confocal microscopy (20×). Living cells appear in green, dead or injured cells in red. **(B)** Bone tissue stained with calcein-AM/ethidium homodimer-2 before OGD. **(C)** Staining after 7 h of OGD. Scale bar is 100 μm.
HAS: Hyperacute serum; OGD: Oxygen–glucose deprivation; PRP: Platelet-rich plasma.

with a live/dead fluorescent kit (Calcein/Ethidium-Homodimer) and examination under confocal microscopy (Figure 2).

Growth factor profile & laboratory parameters

Growth factors and angiogenesis-related proteins in the serum fractions were quantified using the Proteome Profiler Human Angiogenesis Array Kit (R&D System, #ARY 007) according to the manufacturer's instructions. Individual protein levels were measured as the spot intensity on the blots using Adobe Photoshop software and expressed in arbitrary units. All imaging parameters were held constant for averaging and comparison among experiments. Each sample on the dot-blot was performed in duplicates and the complete blot was performed three-times with three different donor samples.

A Sysmex XT-4000i cell counter was used for the quantitative determination of platelet number in the serum fractions. Total protein, albumin and IgG were measured using a Beckman Coulter AU5800 automated laboratory machine (n = 3 HAS samples and n = 2 samples each of native PRP and PPP).

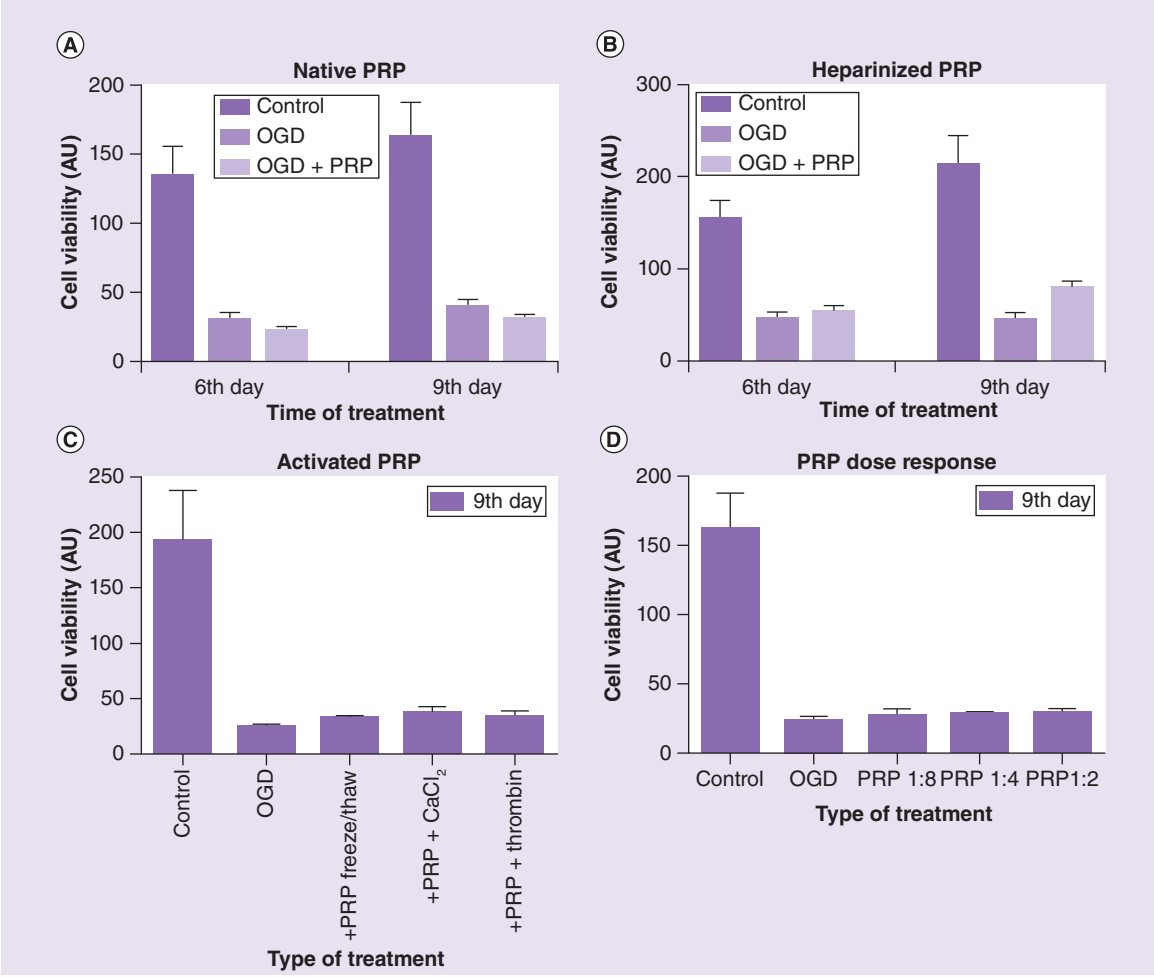

Figure 3. Platelet-rich plasma did not protect bone explants from ischemic degeneration. Neither native PRP **(A)** nor heparinized PRP **(B)** had any effect on viable cell number 3 and 6 days post-OGD (n = 18 per group). **(C)** Similarly, activated PRP had no effect on cell viability (n = 12 per group). **(D)** Increasing the concentration of PRP to the technically feasible maximum level without affecting cell viability through media dilution had no effect on proliferation (n = 6 per group).
OGD: Oxygen–glucose deprivation; PRP: Platelet-rich plasma.

Statistical analysis

Results are expressed as mean ± SEM. Treatment group means (e.g., control, PRP and HAS) were compared by one-way analysis of variance (ANOVA) with Tukey's post hoc tests for pair-wise comparisons. Two-way ANOVA and Bonferroni correction were used to compare results of growth factor profiles. Recombinant growth factors were analyzed with one-way ANOVA and Dunnett test. A $p < 0.05$ (two tailed) was considered significant for all tests. All analyses were conducted using GraphPad Prism software.

Results

Oxygen–glucose deprivation reduced bone cell proliferation rate in explant culture

Bone explants were covered in live cells by the third day in culture (Figure 2B). 7 hours of OGD resulted in significant cell damage as evidenced by live/dead cell staining (Figure 2C). Furthermore, there was a roughly 80% reduction in viable cell number on culture day 6 (3 days postischemia) and little subsequent increase by culture day 9 (6 days postischemia), indicating that surviving cells had reduced proliferation capacity (Figure 3, control conditions).

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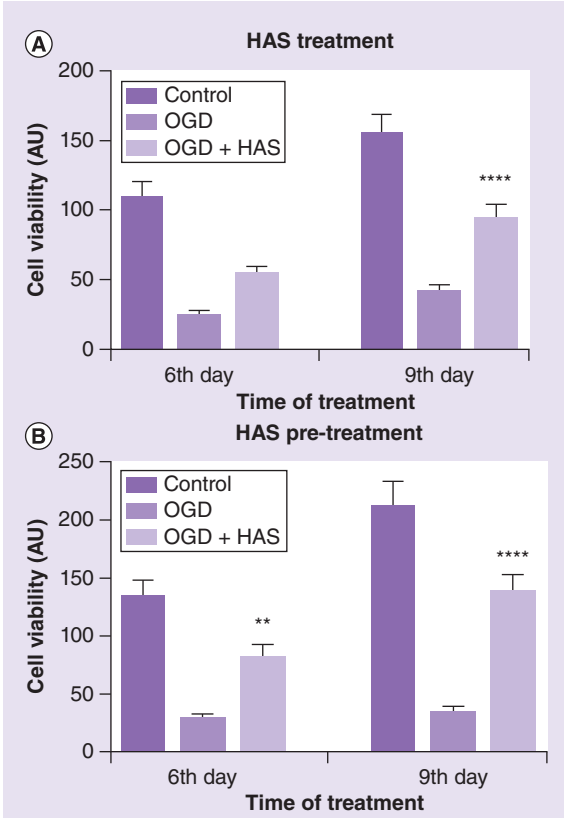

Figure 4. Hyperacute serum reversed post-ischemic degeneration by restoring proliferative capacity. (A) Post-treatment with HAS had no effect on viable cell number immediately after OGD (not shown), but enhanced cell number 3 and 6 days post-OGD (days 6 and 9 *ex vivo*, n = 30 per group). This proliferation-inducing effect was even more pronounced when HAS was added before OGD (n = 24 per group) **(B)**. **p < 0.01, ****p < 0.0001 versus the OGD group. HAS: Hyperacute serum; OGD: Oxygen–glucose deprivation.

Damage from OGD was not rescued by PRP

Addition of native (Figure 3A) or heparinized PRP (Figure 3B) to the cell culture medium failed to reverse the reduction in viable cell number or restore proliferative capacity following OGD (n = 18 samples per group). Similarly, neither PRP releasate prepared by four freeze–thaw cycles nor platelet activation (using $CaCl_2$ or $CaCl_2$ plus thrombin) reversed the reduction in cell number or proliferation rate (n = 12 samples per group) (Figure 3C). Increasing the native PRP concentration to a technically feasible maximum also did not influence postischemic cell damage (Figure 3D). As a last resort, PRP was pelleted and added to the culture dish in a concentrated form to avoid media dilution, but this too did not improve the protective efficacy of PRP (n = 6 samples per group) (results not shown).

Hyperacute serum treatment enhances proliferation rate following OGD

The addition of HAS restored the proliferative capacity of bone cells following OGD. Cell viability was significantly higher in the HAS group compared with the untreated OGD group 6 days postischemia (day 9 *ex vivo*) (n = 30; $p < 0.0001$) (Figure 4A). This proliferative effect was even more pronounced when HAS was present in the cell culture media during OGD (Figure 4B). Significant differences between the control OGD and HAS pretreatment groups were found both on day 3 post-OGD (day 6 *ex vivo*; n = 24, $p < 0.01$) and day 6 post-OGD (ninth day *ex vivo*; $p < 0.0001$). However, there was no significant increase in viable cell number immediately following OGD, even if HAS was present during the treatment (data not shown). Thus, the primary effect of HAS appears to be restoration of postischemic proliferation rather than protection against acute damage from OGD.

Cellular & protein contents of HAS & PRP

The HAS and PRP preparations prepared from the same donors had similar protein compositions, and individual protein concentrations fell within the normal expected ranges for blood serum (Table 1). As expected, we observed less fibrin/fibrinogen in the HAS compared with PRP preparations since only the eluate of the clot was used as HAS. Similarly, HAS contained no measurable hemoglobin, red blood cells or white blood cells and platelet count was significantly lower than in PRP and PPP (Table 2). These findings indicate that HAS contains only the releasate of blood cells but no living blood cells.

Table 1. Protein levels of blood derivatives used in the experiments.

Proteins	HAS	PRP	PPP	Ref.	Unit
Total protein	74.87 ± 1.53	75.5 ± 2.10	75.4 ± 2.70	60–80	g/l
Albumin	49.67 ± 0.07	48.5 ± 0.05	48.4 ± 0.30	35–52	g/l
IgG	12.04 ± 1.34	11.68 ± 2.28	11.69 ± 2.35	6.9–14	g/l
Haemoglobin	0.00 ± 0.00	12 ± 3	0.00 ± 0.00	115–155	g/l
Fibrinogen	0.00 ± 0.00	3.2 ± 0.3	3.15 ± 0.35	1.5–4	g/l

Proteins were measured by using standard laboratory hematology devices: Beckman Coulter AU5800 and Sysmex XT -4000i. Reference ranges shown are normal values in whole blood. As expected, both albumin and IgG are preserved in PRP, PPP and HAS, and since these proteins are the main constituents of plasma the total protein level is also comparable. Fibrinogen is basically missing from HAS since it is already coagulated and retained in the clot during HAS preparation. Haemoglobin is probably derived from the red blood cells that are still present in PRP (see Table 2). Data are presented as average ± SEM. n = 3 in samples of HAS and n = 2 in samples of native PRP and PPP.
HAS: Hyperacute serum, PPP: Platelet-poor plasma; PRP: Platelet-rich plasma; SEM: Standard error of the mean.

Table 2. Cell counts of blood fractions.

Cell counts	HAS	PRP	PPP	Ref.	Unit
Red blood cells	0.00 ± 0.00	0.61 ± 0.08	0.00 ± 0.00	4.2–6.1	T/l
White blood cells	0.01 ± 0.01	12.1 ± 1.6	0.00 ± 0.00	4.8–10.8	g/l
Platelets	1.33 ± 0.33	2354.5 ± 90.5	34.5 ± 11.5	150–400	g/l

Cell counts were determined using a Sysmex XT-4000i device. Reference ranges shown are normal values in whole blood. The measurements confirmed what was deduced from the respective preparations of blood derivatives. HAS is basically devoid of cells or platelets, just like PPP. PRP has platelet values higher than in whole blood. Data are presented as average ± SEM. n = 3 in samples of HAS and n = 2 in samples each of native PRP and PPP.
HAS: Hyperacute serum, PPP: Platelet-poor plasma; PRP: Platelet-rich plasma; SEM: Standard error of the mean.

Growth factor profiles of PRP & HAS

PRP and HAS also demonstrated significantly different growth factor profiles, with angiopoietin ($p < 0.0001$), EGF ($p < 0.0001$), heparin-binding EGF (HB-EGF) ($p < 0.01$), PDGF ($p < 0.0001$), VEGF ($p < 0.0001$) and MMP-8 ($p < 0.001$) at higher concentrations in the PRP preparation, but PF-4 ($p < 0.0001$), serpin ($p < 0.0001$) and TIMP-1 ($p < 0.0001$) higher in HAS (Figure 5). Multiple additional blood-borne growth factors were present in both PRP and HAS at similar concentrations (Figure 5).

Regenerative efficacies of recombinant growth factors

We also tested whether recombinant growth factors known to promote bone healing can improve cell viability following OGD. When added individually, BMP-2 ($p < 0.05$), BMP-7 and PF-4 dose-dependently promoted cell proliferation as evidenced by higher viable cell number 6 days post-OGD (Figure 6). PDGF ($p < 0.01$ and $p < 0.05$) also improved cell viability, although the effect was stronger at the lowest and highest doses compared with the medium dose (Figure 6).

Discussion

Although PRP has demonstrated generally positive results in the treatment of degenerative conditions such as knee OA, wider clinical application is limited because of the unpredictability of constituent profile among preparations and lack of protocol standardization [32]. These sources of variability also limit the ability to extrapolate the results of preclinical studies and clinical trials to general practice [33]. Here, we compared the potential regenerative efficacy of these two biologic compounds in a novel *ex vivo* model of ischemic bone degeneration and found that HAS induced reliable cellular level regeneration by rescuing the postischemic suppression of cell proliferation capacity.

Previous studies have shown that PRP and PRF can have markedly different effects despite similar isolation procedures. For instance, Jeong *et al.* [34] noted that a mixture of tooth ash and PRF was more effective at inducing new bone formation than PRP. In another study, gelatine infused with growth factors released from PRF was more effective than PRP at inducing healing of skin defects [35]. The fibrin mesh of PRF is theorized to release growth factors gradually [36], which may improve biological efficacy. Thus, the lack of a fibrin matrix may have limited the efficacy of HAS compared with PRF. However, removal of fibrin enhances the likelihood that any observed effects are due to the active constituents such as growth factors. However, removal of fibrin enhances the likelihood that any observed effects are due to the active constituents such as growth factors. While PRF and HAS appear to contain the factors needed to restore postishemic osteocyte proliferation regardless of fibrin matrix, PRP lacks these factors. This is further supported by our observation that neither heparinized nor native PRP had any proliferative

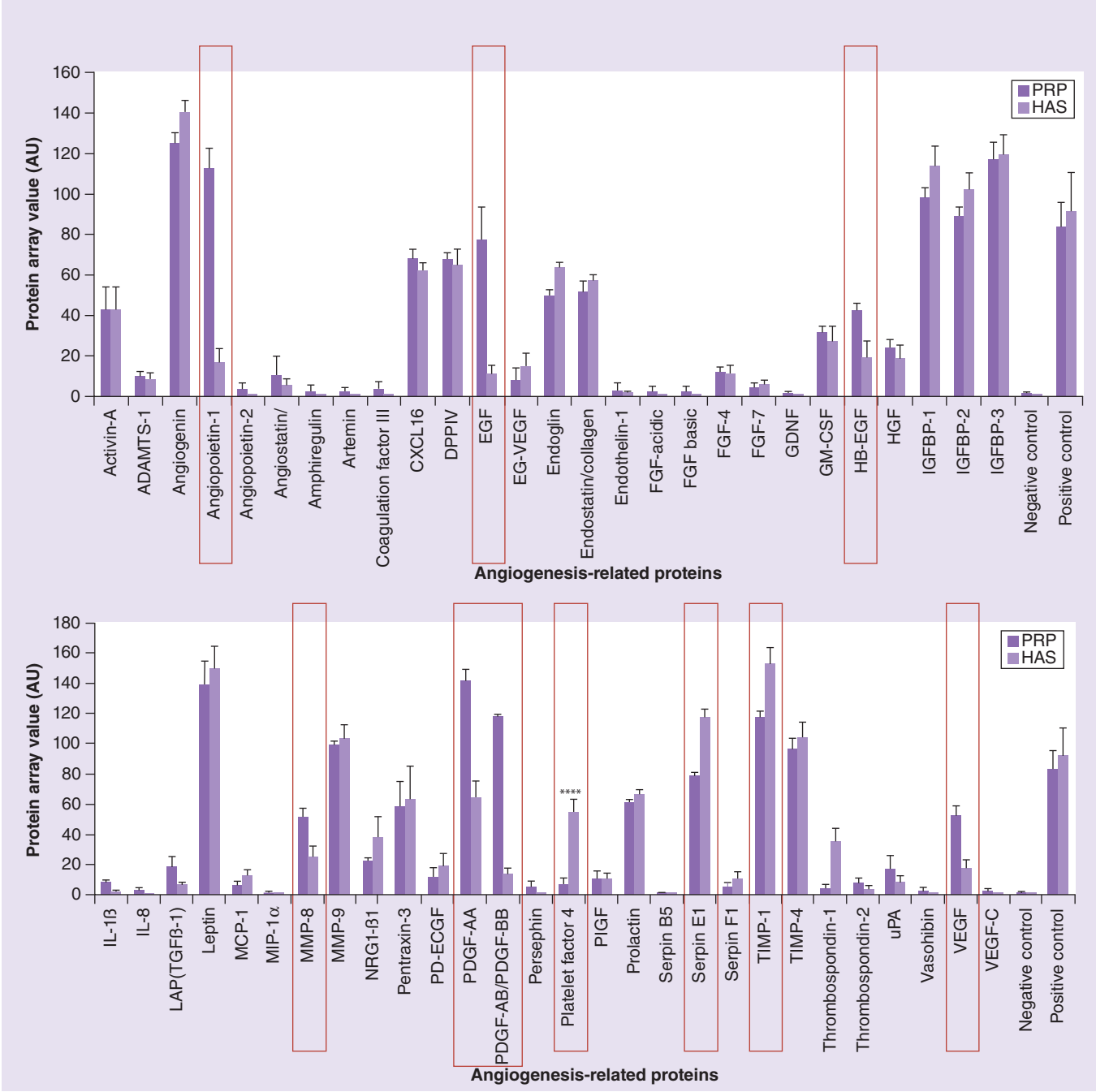

Figure 5. Proteome profiler analysis of platelet-rich plasma and hyperacute serum. Serum preparations were spotted in duplicate to examine the presence of 55 angiogenesis-related proteins. The experiment was reproduced three-times using independent PRP and HAS samples. Relative protein values are shown. Red brackets indicate proteins that differed significantly in concentration between PRP and HAS (**$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$ by ANOVA with Bonferroni *post hoc* test). Results presented as mean ± SEM of three independent experiments, each sample performed in duplicates on the same blots.
ANOVA: Analysis of variance; HAS: Hyperacute serum; PRP: Platelet-rich plasma; SEM: Standard error of the mean.

effect, thus excluding the influence of release kinetics in this specific model. Therefore, the most likely explanation for these observations is a difference in growth factor composition between PRP and HAS. Indeed, there were several key differences in the growth factor profiles between preparations. Although the majority of growth factors were present at comparable levels, the overall pattern suggests that PRP is more angiogenic than HAS [37]. The proteins found at higher concentrations in PRP are angiogenic. Alternatively, PF-4 and thrombospondin 1, which

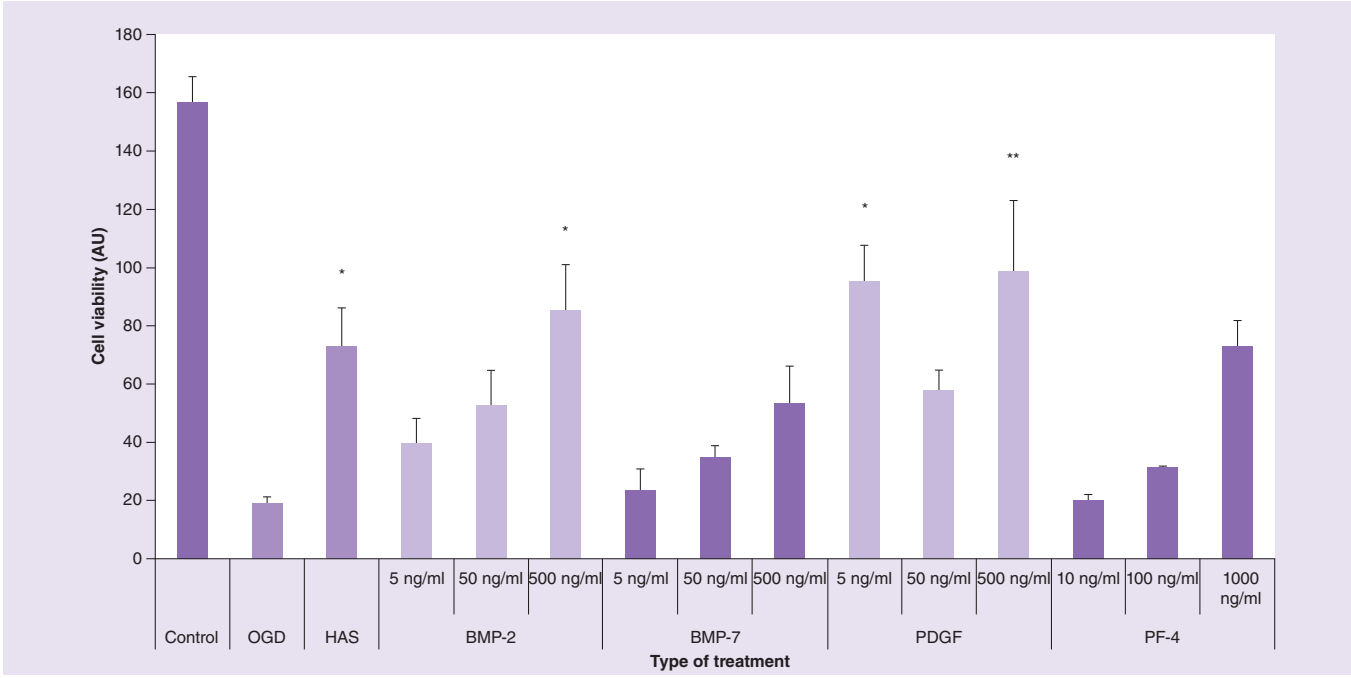

Figure 6. Effect of recombinant growth factors on bone explants 6 days after oxygen–glucose deprivation. Each recombinant protein induced cell proliferation at a level comparable with HAS. *$p < 0.05$ and **$p < 0.01$ versus the control OGD group by ANOVA with Dunnett test.
ANOVA: Analysis of variance; BMP: Bone morphogenic protein; HAS: Hyperacute serum; OGD: Oxygen–glucose deprivation.

are known antiangiogenic factors, were more abundant in HAS. As the key difference between PRP and HAS preparation is the use of anticoagulants, it is possible that arresting coagulation (for PRP) alters the preparation from one antagonistic to angiogenesis to one in which angiogenesis can be induced. The current results showed that only the antiangiogenic growth factor profile supported regeneration of bone cells after simulated ischemic damage. As our experimental design was focused on bone tissue without viable vasculature, further studies are warranted to investigate the angiogenic properties of PRP and HAS in appropriate models.

Recombinant PF-4 alone demonstrated a capacity to induce cell proliferation similar to that of HAS, suggesting that this HAS constituent is responsible for at least a substantial proportion of the overall bioactivity. Indeed, PF-4 is a demonstrated inducer of cell recovery after ischemic damage [38]. The contribution of other factors, however, cannot be discounted as a full proteomics analysis of platelet releasate revealed 3500 proteins, substantially more than can be measured by our antigen-based assays [39,40]. Two other abundant serum proteins, albumin and fibrinogen, are often overlooked in the analyses of factors influencing bone proliferation [41–44]. We have previously described the *in vitro* and *in vivo* bone formation-inducing effects of serum albumin [45,46], which raises the possibility that albumin itself is an active factor in HAS. However, as albumin was present at comparable levels in both serum fractions and was also added to the culture medium as part of FBS in both treatment conditions, it is unlikely that it contributed to the efficacy of HAS. Further, fibrinogen/fibrin is abundant in PRP and absent from HAS, indicating that fibrin alone has little efficacy for enhancing osteocyte proliferation following OGD.

Hyperacute serum improved the proliferation capacity of cells damaged by ischemia to a similar degree as the known inducers of bone regeneration BMP-2 and BMP-7 [47–49]. Moreover, HAS demonstrated regenerative efficacy similar to 500 ng/ml BMP, and the effect was exceeded only by PDGF at this dose. This *ex vivo* evidence is consistent with previous findings that PRF is equally capable of inducing bone growth in an *in vivo* model of rabbit-skull defect healing as PRP and concentrated growth factor [20]. That HAS induced a regenerative effect on par with BMP underscores its potential for treatment of degenerative bone diseases.

This is the first study to use an *ex vivo* human model of bone ischemia to assess the regenerative potential of serum factors. The model used in the present study is not designed to mimic bone healing under normal conditions but rather to reflect the regenerative potential of damaged tissue. Although the inflammatory response following an acute injury to healthy bone may be beneficial, it has the opposite effect in degenerative tissue in

which cell remodeling capacity is impaired. We believe that our current model resembles this latter situation by mimicking a period of ischemia. The observation that serum fractions had no effect on the 'healthy' state of the bone explants (i.e., before OGD) also supports the clinical relevance of the current model for studying degenerative bone. Furthermore, bone samples were harvested from patients with end-stage OA, so the current results can reasonably be interpreted as relevant to OA. Avascular osteonecrosis or aseptic osteonecrosis is actually a bone infarction caused by bone loss after ischemia that eventually leads to severe OA, further supporting the pathogenic response to ischemia-like episodes as a suitable model for degenerative bone disease [50–52].

Conclusion

Our findings indicate that HAS has regenerative properties in damaged bone greater than those of PRP. The fact that HAS is devoid of cells and has generally fewer constituents than PRP but better effects in this specific case suggest this preparation is more amenable to standardization. On the basis of the current *ex vivo* human study, the use of HAS for degenerative conditions such as OA shows great promise, although further studies are required to elucidate the exact mechanisms of action.

Summary points

Osteoarthritis, regenerative medicine & blood derivatives

- Osteoarthritis is a degenerative disease with a significant burden on our society and healthcare expenditure. Regenerative medicine still holds the promise of rebuilding lost tissue functions, however, progress is slower than originally expected. The most widespread clinically applied regenerative agents are blood derivatives, mainly platelet–rich plasma (PRP).
- Blood cells activated by injury secrete a plethora of proliferation factors into the serum. This raises the possibility of using serum products like PRP, platelet-rich fibrin or hyperacute serum (HAS) therapeutically in degenerative bone diseases and other cases of impaired tissue remodeling.
- The exact mode of action of blood derivatives in the osteoarthritic bone tissue is yet unclear.

Our hypothesis

- In the present study, we used a novel *ex vivo* human model of bone ischemia, which closely resembles the pathology of tissue damaged by end-stage degenerative diseases.
- We tested HAS and compared its biological effects to various PRP protocols. We hypothesized that the concentrated platelet-derived will have the most proliferative effect on the ischemically challenged bone tissue.

Materials & methods

- Osteoarthritic subchondral bone pieces were harvested from discarded femoral heads during hip replacement operations. The explants were grown in culture for 3 days then subjected to transient oxygen glucose deprivation (OGD) for 7 h.
- PRP, HAS and recombinant growth factors were added to bone explants undergoing OGD and MTT assay was performed to quantified cell viability at 24 h after OGD.
- Proteome Profiler Human Angiogenesis Array Kit was applied for determination of the growth factors and angiogenesis-related proteins in blood derivatives.

Results

- Adding PRP either native or heparinized, or activated by Calcium, thrombin or physical means did not have any effect on the postischemic cells. However, HAS restored cell proliferation capacity.
- Proteome-profiler analysis showed that PRP and HAS have diverging growth factor profiles. Angiopoietin, EGF, PDGF, HB-EGF, VEGF and MMP-8 were more abundant in PRP, PF-4 (PF-4), Serpin E1 and TIMP-1 were more abundant in HAS, while 17 other factors were at comparable levels.
- Recombinant growth factors like BMP-2, BMP-7, PDGF and PF-4 have strong effects on bone proliferation at a comparable level to HAS but only at the highest doses.

Conclusion

- Our results indicate that HAS significantly improved the proliferative capacity of cells damaged by ischemia, while PRP did not have a positive effect, even at high concentrations, and thus HAS can be a new therapeutic tool in degenerative bone diseases.
- Despite the high growth factor and cytokine content of PRP, it failed to induce proliferation of ischemically damaged subchondral bone tissue, however, the more physiological hyperacute serum showed a positive effect.

Financial & competing interests disclosure

Z Lacza owns stock in a start-up company OrthoSera GmbH that holds patents on HAS. I Hornyak and Z Lacza are partly employed by OrthoSera. The authors do not declare any conflict of interest. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Authors' contributions

All authors make substantial contributions to conception and design and interpretation of data. Z Lacza conceived of the presented idea. B Major and G Vácz developed the theory and planned the experiments. B Major, D Gaál, L Petrik, T Holczer, M Simon and G Vácz performed the measurements and analyzed the data. DB Horváthy and I Hornyák helped supervise the project. Z Lacza wrote the manuscript with support from W Han and JM Muir.

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