Synergy of human platelet lysate and laminin to enhance the neurotrophic effect of human adipose-derived stem cells

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Despite the spontaneous regenerative capacity of the peripheral nervous system, clinical nerve repair often results in poor functional recovery with the high socio-economic burden. In presence of peripheral nerve injuries and when distal nerve transfers are not possible, nerve autografts, are the solution of choice to cross a critical nerve gap. However, these are associated with donor site morbidity and offer only suboptimal functional recovery. Thus, the need to improve the rate of effective regeneration has directed research attention towards stem cell therapy and nerve tissue engineering.

Considering the therapeutic potential of human adipose-derived stem cells (hASC), in particular for peripheral nerve repair, we established a complete xenofree protocol for cell culture and expansion towards clinical translation. Applying human platelet lysate (hPL) in the pre-expansion steps of hASC could improve cell performance for supporting neurite outgrowth and lead to an increased neurotropism when compared with the effect elicited by the same cells grown with fetal bovine serum (FBS). The synergy discovered with the extracellular matrix molecule (ECM) molecules specifically laminin (LN) is particularly promising in long-gap nerve injuries, where the regenerating stumps will need complex requirements for targeted regeneration. hPL-stimulated hASC ($hASC^{hPL}$) showed increased responsiveness and synergy with LN, not only when in direct contact with neurons, but also when neurons were treated with hASC derived secretome on LN substrates. Further, *in vivo* investigation applying $hASC^{hPL}$ to LN-functionalized conduits to cross a critical nerve gap model will be needed to validate the *in vitro* findings, and to move a step forward in the process of clinical transition.

Peripheral nerve injuries are a common clinical problem leading to sensory and motor disability. Despite the spontaneous regeneration capacity of the peripheral nervous system and microsurgical advancements in nerve repair and nerve transfers, the results remain far from optimal. Nerve autografts, which are considered the gold standard in clinical practice, possess important limitations such as donor site morbidity and modality mismatch. The need to improve the rate of effective regeneration has directed clinical and experimental researchers towards stem cell therapy and nerve tissue engineering.

A timely and effective regeneration is primarily influenced by the regenerative niche, which includes growth factors, permissive extracellular matrix scaffolds, and Schwann cells (SC) (Chen et al., 2007). SC are critical to initiate Wallerian degeneration and support axonal pathfinding. Several studies have shown the beneficial effects of transplanted SC for nerve regeneration, however, these cells require rather complex culture conditions and, most importantly, the sacrifice of a functional nerve from the patient, limiting their clinical translation. Thus, the need for viable neurotrophic cell sources such as stem cells emerged.

ASCs have gained researchers' attention because of easy access, fast growth in culture, and the low immunogenic profile. Thus, ASCs became attractive for clinical translation. Moreover, they can be differentiated in SC-like cells, supporting nerve regeneration both *in vitro* (Kingham et al., 2007) and *in vivo* (di Summa et al., 2011; Di Summa et al., 2018), as we reported earlier. However, cell differentiation into SC-like cells is less effective in human ASC and the 3-week extended stimulation with exogenous factors limits potential clinical use. Moreover, SC-like cells revert to their original phenotype once growth factors supplementation is suspended, questioning the role and the necessity of cell differentiation (Faroni et al., 2016), while confirming the need for simplification when it comes to cell therapy and clinical translation.

The role of the extracellular matrix: The peripheral nervous system is capable of intrinsic regeneration capacity, but exogenous trophic and topographical support is critical, particularly over longer gaps. The ideal interactive microenvironment should be engineered to mimic the native tissue, able to influence the function and fate of endogenous, recruited and transplanted cells. The ECMs are key players of such microenvironment: we previously demonstrated how rodent ASCs cultured on ECM such as fibronectin (FN) and LN could significantly enhance neurite outgrowth of dorsal root ganglion (DRG) during cocultures. These molecules provided in fact a rich environment for promoting cell adhesion and migration, by regulating the production, release and functioning of biochemical factors such as growth factors and cytokines (di Summa et al., 2013).

With these observations in mind, we investigated the synergy of ASC (in their undifferentiated state) and LN, as key extracellular matrix molecules, in a 3D *in vitro* co-culture system and *in vivo* delivering the cells into a sciatic nerve gap injury after encapsulation into a fibrin gel.

In the presence of ECM proteins within the gel, we observed a significant increase of fibers' sprouting at the proximal stump, and significantly denser nerve fibers at the distal stump compared to control groups. The cumulative positive effect of fibrin gel-encapsulated ASC and LN in this *in vivo* study developed promising perspectives for using ASC in peripheral nerve repair, as transplanted cells were not pre-differentiated but relied solely on the support of the permissive microenvironment (de Luca et al., 2018).

Improving clinical translation: Aiming for an effective and safe clinical translation, we focused on hASCs combining our experience in regenerative cells with patient safeguarding and optimum safety. Indeed, the risk of infection from animal-derived products is pertinent during cell isolation and culture, and clinical trials evaluating the potential of stem cell therapies are growing faster than research that investigates alternative, xenogeneic-free solutions. While FBS is the most commonly used factor supplement for cell culture could increase the risk of immune reactivity and infection (viral, bacterial and prion) in the recipient patient (Palombella et al., 2020).

For these reasons, we adopted hPL as a viable substitute to FBS since it can be easily obtained, as pooled blood, in large quantitates for clinical applications. When systematically reviewing the literature, hPL supplement showed to support cell viability, enhancing proliferation, while ensuring cell genomic stability and preserving cellular immunophenotype, even in advanced cell passages (Guiotto et al., 2020).

Neurotrophic properties of human platelet lysate: Noteworthy, hPL is known for containing a wide variety of growth factors, including neurotrophin 3, nerve

growth factor (NGF), brain-derived growth factor (BDNF), and glial cell-line derived neurotrophic factor. These molecules are involved in SCs survival, differentiation, ECM deposition, and neurotrophic factor release, all of which will provide a permissive environment for axonal growth and pathfinding (Lischer et al., 2020; Palombella et al., 2020).

When comparing hASCs cultured with either FBS or HPL, we found that metabolic activity of hASCs expanded with hPL was enhanced when compared to FBS-cultured cells, in alignment with their increased proliferation rate. Interestingly, hPL-cultured cells ($hASC^{hPL}$) were not committed to SC-like cells, similarly to FBS-cultured cells ($hASC^{FBS}$), resulting in negative for specific Schwann cells markers such as nestin and glial fibrillary acidic protein, despite the variety of growth factors (GF) present in the hPL serum. However, levels of GF secretion of $hASC^{hPL}$, were significantly higher when compared to FBS-cultured cells, suggesting a direct effect of hPL on the hASC secretome.

Interestingly, BDNF levels in the hPL-conditioned medium were significantly lower than in hPL supplement alone, suggesting that hASC used the growth factors contained in hPL supplement. This was consistent with the BDNF/NTRK2 axis, regulated by negative feedback (and in line with PCR results showing low expression of NTRK2 receptor on $hASC^{hPL}$). Thus, the abundance of GF (e.g., BDNF) coming from hPL possibly inhibited the expression of NTRK2 in the hASC to prevent an over-uptake. Conversely, a lower detected level of NGF in hPL medium alone, but a higher one in hPL-conditioned medium, even if not significantly, could be the result of an increased hASC secretion of the GF, in line with a similar expression profile of its receptors (NGFR and NTRK1) (Palombella et al., 2020).

These preliminary data indicate that hPL grown-hASC regulate their GF intake and their receptor expression and, to an extent, may induce a potential release of accumulated GFs when transplanted *in vivo*.

To functionally prove a potential neurotrophic modulation in $hASC^{hPL}$, we established functional cocultures in both chicken embryonic (Lischer et al., 2020) and rat DRG coculture models (Palombella et al., 2020). DRG organotypic explants were put directly in contact with $hASC^{hPL}$ or with $hASC^{hPL}$ secretome only, in order to analyze the functional outcomes observed in presence of cell-to-cell interactions or with released factors only.

$hASC^{hPL}$ derived secretome was shown to have a statistically significant effect on the regeneration of the DRG neurons, by promoting an increased maximal neurite length and neurite extension area. Overall, the direct contact between the $hASC^{hPL}$ and the DRG supported even higher neuronal regeneration as evidenced by their impact on neurite maximal length and neurite extension area, when compared to $hASC^{FBS}$ conditions.

Importantly, when $hASC^{hPL}$ were shifted to a serum-free condition at the moment of DRG co-culture, the supportive nature of these cells with respect to nerve regeneration was maintained, while no improved outgrowth was recorded $hASC^{FBS}$ (Lischer et al., 2020; Palombella et al., 2020). These observations represent a crucial finding, suggesting that, once stimulated by hPL, hASC could maintain a neurotrophic potential without further serum supplementation, which may be an ideal feature for *in vivo* transplantation, where cells get exposed to anoxic and serum-free scenarios.

The synergy between laminin and $hASC^{hPL}$ in DRG functional assays: When facing critical nerve gaps *in vivo*, directional cues are a landmark of bioengineered nerve conduits and permissive ECM scaffold had shown in our previous work to be critical for enhanced and targeted regeneration.

LN, as the main basal lamina component, is a fundamental component of the ECM both in the central and the peripheral nervous systems, supporting a variety of functions including SCs migration, axonal outgrowth, and remyelination. FN is secreted by glial cells promoting cell growth, survival, and proliferation. Both support the recovery after nerve injuries: the former (LN) stimulating axonal elongation and activating SCs in myelin production, the latter (FN) increasing neural cell adhesion and SC proliferation (di Summa et al., 2013).

Building on our previous works, and in order to define the better suited and permissive coating for a future bioengineered conduit, we investigated the impact of $hASC^{hPL}$ in combination with different ECM-molecule-coated surfaces, and we studied their individual and combined potentials to improve neurite outgrowth in a DRG explant model (Guiotto et al., 2021).

The first key result was that the supplement alone (hPL or FBS), without the presence of hASC, was not able to influence DRG neurite outgrowth. This confirmed that neurotrophic effects are not simply a consequence of a higher concentration of trophic molecules in the medium, but require cell-mediated responses for a natural neural support. On the other hand, the ECM impact on DRG outgrowth showed meaningful variation when explants were cultured on LN-coated surfaces. These findings suggest different roles for the medium additive and the ECM scaffolds: if the first plays more an effect of cell stimulation and induction of a secretome, the second actively enhances neurons interaction, axonal sorting, and directionality.

When preconditioned $hASC^{hPL}$ in the presence of LN substrate were placed in contact with DRG, neurite extension and axonal area were superior to any other condition (including FN substrates), suggesting the synergy between the hPL cell preconditioning and the matrix support to both hASC and neurons. As indicated above, when focusing specifically on the secretome, DRGs neurons were grown with $hASC^{hPL}$ conditioned medium only: data showed a significant difference between the $hASC^{FBS}$ and $hASC^{hPL}$ secretome where the latter increased neurite length and density, on either ECM coated surfaces (LN, FN) but not on tissue culture plastic. These observations suggest a significant role of the secretome-matrix-neuron interaction network. Particularly, on LN, primary neurons evidenced the highest response to the $hASC^{hPL}$ secretome, confirming that LN coating represents part of an ideal environment for regenerating neurons, enabling their response to the neurotrophic effect of the stem cells secretome and sustaining axonal elongation (Guiotto et al., 2021).

Different components seemed therefore to act in a crucial interplay to support regeneration: cells expanded on hPL present a stronger proliferation and guarantee an attractive secretome for neuronal cultures; on the other hand, the ECM component LN, acts both on hASC and neurons facilitating adherence and cell-cell interactions.

hASC modifications under hPL stimulation and future perspectives: In a recent work, we investigated by transcriptional and phosphoproteinomics analysis the molecular changes taking place when hASC were stimulated by different neurotrophic factors (Prautsch et al., 2020). Exogenous neurotropic factor stimuli may amplify hASC activities through their receptor binding, with phosphorylation of different proteins involved in growth factors signaling, leading to the enhanced expression of regeneration-associated molecules, cytokines and growth factors. In the specific case of hPL-related hASC stimulation, we could find an increased release of GFs such as NGF and BDNF, maintaining such features despite the removal of serum after 48 hours of treatment, with clear neurotrophic effects on primary neurons. Moreover, the synergia discovered with the ECM component LN is particularly promising in long-gap nerve injuries, where the regenerating

stumps will need exogenous support for targeted regeneration. hPL showed to be able to guarantee, besides enhanced hASC proliferation and maintenance of cell plasticity, a more efficient cell secretome when compared to FBS standard supplement. hASC^{hPL} showed increased responsiveness and interactivity with LN, not only when in direct contact with neurons, but also when neurons were treated only with hASC^{hPL} secretome on LN substrates (**Figure 1**).

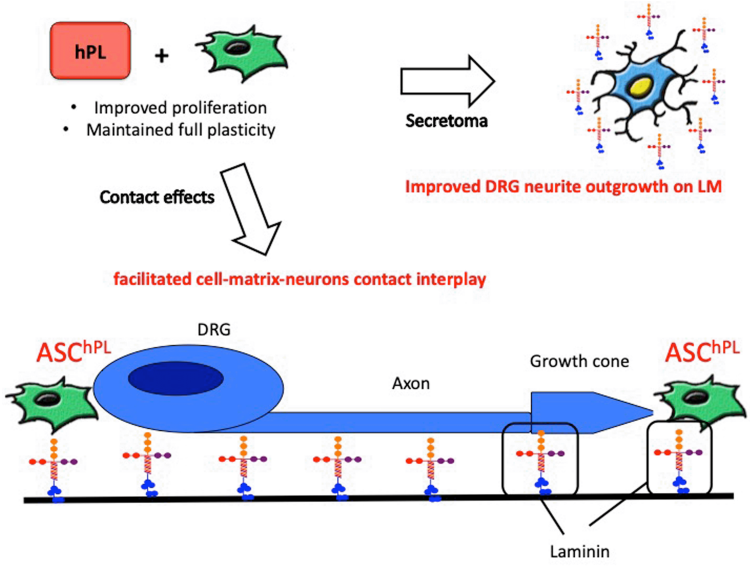

Figure 1 | **Illustration on potential interplay between hASC^{hPL}, laminin, and primary neurons.**
The effects of the hPL additive lead to an improved cell secretoma and an enhanced cell activity when in contact with neurons. Such effects were further multiplied by the presence of laminin in a synergic way. ASC: Adipose-derived stem cells; DRG: dorsal root ganglia; hPL: human platelet lysate; LM: laminin.

Mechanisms of such interplay remain elusive, considering the number of actors (hPL and its different components, hASC, ECM, neurons) and the complexity of their interactions. Functional cocultures with neurons showed evidence of how the synergia between hPL stimulation and matrix support could multiply the neurotrophic effects. Further investigations at the cell and molecular level are needed for a better understanding of the hASC^{hPL} secretome and of cell-matrix-neurons interactions.

Further, *in vivo* investigation using bioengineered nerve grafts endowed with LN-functionalized surface structures and hASC^{hPL} in the context of critical nerve gap injury model will be needed in order to move a step forward with the clinical transition of xenogeneic free therapeutic strategies (i.e., hPL).

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References

Chen ZL, Yu WM, Strickland S (2007) Peripheral regeneration. Annu Rev Neurosci 30:209-233.
de Luca AC, Fonta CM, Raffoul W, di Summa PG, Lacour SP (2018) In vitro evaluation of gel-encapsulated adipose derived stem cells: Biochemical cues for in vivo peripheral nerve repair. J Tissue Eng Regen Med 12:676-686.
di Summa PG, Kalbermatten DF, Raffoul W, Terenghi G, Kingham PJ (2013) Extracellular matrix molecules enhance the neurotrophic effect of Schwann cell-like differentiated adipose-derived stem cells and increase cell survival under stress conditions. Tissue Eng Part A 19:368-379.
di Summa PG, Kalbermatten DF, Pralong E, Raffoul W, Kingham PJ, Terenghi G (2011) Long-term in vivo regeneration of peripheral nerves through bioengineered nerve grafts. Neuroscience 181:278-291.
Di Summa PG, Schiraldi L, Cherubino M, Oranges CM, Kalbermatten DF, Raffoul W, Madduri S (2018) Adipose derived stem cells reduce fibrosis and promote nerve regeneration in rats. Anat Rec (Hoboken) 301:1714-1721.
Faroni A, Smith RJ, Lu L, Reid AJ (2016) Human Schwann-like cells derived from adipose-derived mesenchymal stem cells rapidly de-differentiate in the absence of stimulating medium. Eur J Neurosci 43:417-430.
Guiotto M, Raffoul W, Hart AM, Riehle MO, di Summa PG (2020) Human platelet lysate to substitute fetal bovine serum in hMSC expansion for translational applications: a systematic review. J Transl Med 18:351.
Guiotto M, Raffoul W, Hart AM, Riehle MO, di Summa PG (2021) Human platelet lysate acts synergistically with laminin to improve the neurotrophic effect of human adipose-derived stem cells on primary neurons in vitro. Front Bioeng Biotechnol 9:658176.
Kingham PJ, Kalbermatten DF, Mahay D, Armstrong SJ, Wiberg M, Terenghi G (2007) Adipose-derived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth in vitro. Exp Neurol 207:267-274.
Lischer M, di Summa PG, Oranges CM, Schaefer DJ, Kalbermatten DF, Guzman R, Madduri S (2020) Human platelet lysate stimulated adipose stem cells exhibit strong neurotrophic potency for nerve tissue engineering applications. Regen Med 15:1399-1408.
Palombella S, Guiotto M, Higgins GC, Applegate LL, Raffoul W, Cherubino M, Hart A, Riehle MO, di Summa PG (2020) Human platelet lysate as a potential clinical-translatable supplement to support the neurotrophic properties of human adipose-derived stem cells. Stem Cell Res Ther 11:432.
Prautsch KM, Schmidt A, Paradiso V, Schaefer DJ, Guzman R, Kalbermatten DF, Madduri S (2020) Modulation of human adipose stem cells' neurotrophic capacity using a variety of growth factors for neural tissue engineering applications: axonal growth, transcriptional, and phosphoproteomic analyses in vitro. Cells 9:1939.

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