While many international clinics are concentrating on providing patient therapy with autologous adult stem cells, one of the issues that we have seen is the need to use large numbers of stem cells to achieve a therapeutic result. At the Drake Biomedical Institute, we have set this number arbitrarily at $1 \times 10^9$ cells/ml. Most clinics use far fewer stem cells because practically speaking, it is difficult to harvest high numbers with a single blood sample. This has led to concerns about how to multiply stem cell numbers in a clinical setting. One aspect of patient stem cell therapy that has not received enough attention is: Growth factors...that is, what cytokines, proteins, messenger molecules and other extracellular biologicals can be used to boost expansion of stem cells ex vivo, and how to go about isolating and describing more substances that can aid in expansion protocols.

1. The Paired Mice Experiments: The Paired Mice experiments demonstrated that serum factors rather than transplanted stem cells caused enhanced muscle regeneration in aged mice. The way the experiment was done, an old mouse is surgically conjoined with a young mouse in such a way that their circulatory systems are mixed, and blood, plasma, and serum flows between them. Although for our purposes, the seminal paper was issued over 10 years ago in 2005, the importance of the paired mice experiments has yet to be fully appreciated by clinicians, physicians, and clinical researchers all attempting to advance Adult Stem Cell therapy, and in particular, autologous stem cell therapy (cells harvested from the patient to be treated).

The researchers observed that after conjoining, the stem cells in the old mouse were activated to begin dividing again, and that muscle repair in the old mouse was enhanced and became similar to that of young mice.

By marking cells in the young mouse, the experiment further demonstrated that the improvement in the old mouse muscle repair was not due to stem cells from the young mouse, but rather due to circulating blood factors. In addition, it was found that one could replicate the finding in vitro, by just adding plasma from young mice to stem cells of old mice in culture. Irina M. Conboy, Michael J. Conboy, AmyJ. Wagers, Eric R. Girma, Irving L. Weissman, Thomas A. Rando, “Rejuvenation of aged progenitor cells by exposure to a young systemic environment”, Nature 433:760 (2005).

In 2008, part of the same team, in a follow-up work, better identified some of the serum factors involved, noting that serum from the young mice activates the Notch pathway in the older animals, and also results in the deactivation of Transforming growth factor (TGF-β). Carlson, ME, “Imbalance between pSmad3 and Notch induces CDKinhibitors in old muscle stem cells”, Nature 454:528 (2008). In 2014, they further identified the circulating hormone oxytocin, that when injected into aged mice regenerates muscles by activating muscle stem cells. Elabd,C et al “Oxytocin is an age-specific circulating hormone that is necessary for muscle maintenance and regeneration” Nature Commun. 5:4082 (2014)

As summarized in the article “Ageing research: Blood to blood”, [Megan Scudellari, Nature 517:426-429 (2015): “Wyss-Coray, who worked in the room next to Rando’s lab, had previously discovered prominent changes in levels of proteins and growth factors in the blood of ageing
humans and people with Alzheimer’s disease. Following up on Rando’s unpublished brain results, he used old–young mouse pairs to show that old mice exposed to young blood did indeed have increased neuron growth, and that young mice exposed to old blood had reduced growth. Plasma alone had the same effects. “We didn’t have to exchange the whole blood,” says Wyss-Coray. “It acts like a drug.” Villeda, SA et al, “The ageing systemic milieu negatively regulates neurogenesis and cognitive function”, Nature 477:90 (2011).

2. The number of stem cells may remain high as we age. This is a point still being debated, but a lot of data now shows that we may not lose stem cell numbers as we age, but rather, the regenerative function of the stem cells declines as we age, and that this is due to changes in cellular environment. Take for example this quote from an important review paper:

> “Recent evidence supports the model that stem cells in several tissues are largely retained in a quiescent state but can be coaxed back into the cell cycle in response to extracellular cues, even after prolonged periods of dormancy.” Sharpless NE, and DePinho RA, “How stem cells age and why this makes us grow old”, Nature 8:703 (2007).

Bottom line, many international clinics are just implanting more stem cells, and while the results are noteworthy, we could probably do a whole lot better by adding known stem cell activators to an expansion protocol prior to transplantation of the stem cells.

3. Known and unknown Growth Factors. Only a small handful of growth and activating factors that can be used to stimulate expansion ex vivo are known:

- Transforming Growth Factor (TGF-β)
- Insulin Growth Factor (IGF)
- Interleukin 3 (IL-3)
- Interleukin 6 (IL-6)
- Stem Cell Factor (SCF)
- Granulocyte Colony Stimulating Factor (G-CSF)
- Megakaryocyte Growth and Differentiation Factor (MGDF)
- Stromal Cell Derived Factor (SCDF)

Note that we are not concerned with all the many substances under study or elucidated relating to the manipulation of *embryonic* stem cells in vitro, as these substances used in these artificial models appear to have little applicability to the clinical treatment with *adult* stem cells.

In addition, although described in lesser detail and more so in terms of “fractions” of blood, we know that Endothelial cells, and endothelial cell components, plasma fractions, lysates, and other portions of a total blood sample contain powerful, though ill defined, additional stimulatory and growth activators. In some cases, we can refer to some of these as the vascular fraction or the stromal vascular fraction of whole blood. For example, Dr. Christopher Centeno of the Centeno-Schultz Orthopedic Clinic which treats patients with adult stem cells, believes that growth factors from the patient’s own platelets are required to better expand autologous stem cells for reimplantation to the patient. That is, platelets stimulate expansion, even though the exact factors are not identified. [http://centenoschultz.com/incoming-patients/published-research-articles/](http://centenoschultz.com/incoming-patients/published-research-articles/)

**Conclusion**

The paired mice experiments are troubling in that they show that serum factors, not the transfer of stem cells themselves, is what caused marked improvement in ability to heal injury in aged mice.
Therefore, in order to really become expert in using adult stem cells for therapeutic use, it would be nice to have an arsenal of cytokines, hormones, small molecules, etc, that could be called upon in specified situations. Not necessarily off the shelf products, which may not be clinically useful unless they could be synthesized to avoid patient contamination. If we knew how to easily isolate the required growth factors and activating factors from a patient’s blood, then those substances could be used in tandem with autologous stem cell transplants to further boost the usefulness of overall stem cell therapy.