Regenerative medicine offers much promise for those suffering from several disease states. It is a method of using your own natural healing properties to assist in living a better quality of life. Stem cells are presently the largest target in the regenerative medicine arena.

Stem cells are naïve cells that have the ability to divide indefinitely. They may functionally change into any cell in our body and can integrate to the target tissue, either in the presence or absence of damage. Stem cells may be isolated from several locations including the blastocyst, fetal tissue (various organs of such), umbilical cord blood/tissue and adult tissues (bone marrow, liver, fat, brain, muscle, and other tissues). There are various classifications of stem cells.

1. Totipotent, able to produce all embryonic and extra-embryonic tissue, i.e., all tissues that form us.
2. Pluripotent, which have the ability to change into any tissue from all three embryonic layers except placenta.
3. Multipotent, which have the ability to change into two or more different cell types.
4. Unipotent, only give rise to one cell type.

We can find stem cells throughout development and in our adulthood. In adulthood, they are easily access from our bone marrow and fat tissue. Adult stem cells are considered multipotent stem cells. There are many drawbacks to using your own stem cells. First, to remove stem cells from your body requires a surgical procedure, which as in every procedure, carries some form of risk, pain, and discomfort. Second, stem cells age as we do and their numbers decline substantially with age. Finally, there is scientific evidence illustrating that stem cells from a disease patient are not as effective as those from a healthy one.

There are different types of stem cells in our bodies. There are hematopoietic stem cells (HSCs) or blood stem cells as these are the stem cells that give rise to all components of blood. The other stem cell is called a mesenchymal stem cell (MSCs). These particular stem cells have shown much promise in various disease states as they give rise to a multitude of cell types found throughout our body such as fat, bone, cartilage and muscle.

MSCs have the ability to migrate and target specific tissues. This property called homing is an event that allows cells to migrate from a remote area in the body to find a damaged organ or tissue in a specific site. This is the mechanism by which MSCs are infused intravenously and reach the affected areas of the body to perform its regenerative functions.

Experimental models have shown that MSCs have several important functions. They are able to regenerate damaged or injured tissues. In vitro and in vivo studies illustrate the plasticity of MSCs ability to give rise to non-hematopoietic cells such as myocytes, tenocytes and nerve cells. MSCs can modulate immune responses because of their specific properties. They are non-immunogenic cells that express few major histocompatibility complex class I antigens (MHC I) and do not express either major histocompatibility complex class II antigens (MHC II) or co-stimulatory molecules, rendering them incapable of activating a T-cell response. They regulate the immune system by increasing the response of regulatory T-cells and decreasing pro-inflammatory mediators such as TNF-α, and IFN-γ. Thus, MSCs may be used from an unrelated donor. MSCs have been approved for pediatric graft vs host disease and are in clinical studies for adult indications of such. The use of MSCs for graft vs host disease clearly demonstrates that MSCs are safe for allogeneic use.

MSCs work in a paracrine manner to aid in host endogenous repair. MSCs release growth factors and proteins to communicate and effect neighboring cells. Studies illustrate a host of cytokines, chemokines and growth factors released by MSCs such as VEGF, FGF, PDGF, SCF to name a few. Growth factor release such as that of VEGF helps in the formation of new vascularization or angiogenesis while release of IL-1ra aid in suppressing the pro-inflammatory response of TNF-α. Hence, MSCs work in various methods to aid in healing and natural repair.

Liveyon regenerative medicine product contains stem cells and growth factors which may be beneficial for repair, growth and healing. Here independently completed in vitro studies illustrate the quantity of cells and cell surface markers indicative of MSCs obtained from Liveyon regenerative medicine product. Most importantly, the release of 4 growth factors that aid in the formation of new blood vessels, activation of endogenous stem cells, modulation of the immune system and growth of cells and tissue.
RESULTS

Liveyon regenerative medicine product was delivered and maintained at -80°C until ready for testing. Various vials of regenerative medicine product were tested with consistent results as described below in (Fig 1a-b). Product was thawed using a passive thaw method where the individual just placed the frozen vial into their hand and thawed the product.

Once product was in a liquid form it was evaluated. Average time of thaw took approximately 3-4 minutes. An aliquot of Liveyon regenerative medicine product was taken and stained using acridine orange (AO) and propidium iodide (PI) followed by quantification using the Nexcelom Cellometer Auto 2000.

Assay: DV Primary Cells

Sample ID: Liveyon Passive Thaw (from vial) 12.19.16
Dilution Factor: 2.00

<table>
<thead>
<tr>
<th>Count</th>
<th>Concentration</th>
<th>Mean Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total: 5147 cells</td>
<td>1.78x10^7 cells/mL</td>
<td>6.2 microns</td>
</tr>
<tr>
<td>Live: 3053 Cells</td>
<td>1.05x10^7 cells/mL</td>
<td>5.8 microns</td>
</tr>
<tr>
<td>Dead: 2094 Cells</td>
<td>7.24x10^6 cells/mL</td>
<td>6.7 microns</td>
</tr>
</tbody>
</table>

Viability: 59.3%

Figure 1a: Post Thaw from Vial containing 10 million cells illustrates that > than 10 million cells obtained following thaw.

Assay: DV Primary Cells

Sample ID: Liveyon Blue DT00628/Lot G007-010517 Ct 170126-1422
Dilution Factor: 2.00

<table>
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<tr>
<th>Count</th>
<th>Concentration</th>
<th>Mean Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total: 20047 cells</td>
<td>6.93x10^7 cells/mL</td>
<td>6.7 microns</td>
</tr>
<tr>
<td>Live: 13112 Cells</td>
<td>4.53x10^7 cells/mL</td>
<td>5.8 microns</td>
</tr>
<tr>
<td>Dead: 6935 Cells</td>
<td>2.40x10^6 cells/mL</td>
<td>7.4 microns</td>
</tr>
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</table>

Viability: 65.3%

Figure 1b: Post Thaw from vials containing 30 million cells illustrates > than 30 million cells obtained following thaw.
The remainder of the product was evaluated for flow cytometry using BD Accuri C6 Flow Cytometer (Fig 2). Briefly, the cells in the Liveyon regenerative medicine product were stained with FITC or PE directly conjugated antibodies to CD34, CD45, CD90, HLA-DR and HLA-ABC. These markers were chosen as they are standard markers used for MSCs.

<table>
<thead>
<tr>
<th>CD Marker</th>
<th>Percentage</th>
<th>Total Cells</th>
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<tbody>
<tr>
<td>CD34</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>CD45</td>
<td>72.50%</td>
<td>1.54x10^6</td>
</tr>
<tr>
<td>CD34 in CD45dim</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>CD90</td>
<td>3.20%</td>
<td>6.3x10^4</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>0.24%</td>
<td></td>
</tr>
<tr>
<td>HLA-ABC</td>
<td>76.76%</td>
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</tbody>
</table>

Most importantly is the demonstration of the lack of HLA-DR (MHC-II) which is consistent with MSCs ability to be immune privileged (evasive). CD34 cells were not detected in samples tested.

**Figure 2**: Flow cytometry illustrates the presence of mesenchymal stem cells (MSCS) which are identified by the marker CD90.
Liveyon product was next tested for growth factor release using enzyme-linked immunosorbent assay (ELISA) (Fig 3; Table 1). One vial of cells from 2 donors (583 and 645; lots 120116 and 120216, respectively) was subjected to three cycles of freeze/thaw, and diluted in phosphate buffered saline (PBS); the cell lysate volume was adjusted to 5 ml with PBS and tested.

**Fig. 3.** Cytokine/Chemokine determination in cell lysates. ELISA was performed to detect and quantify FGF-2, VEGF, SCF, and IL-1ra. The average result of each assay is shown. IL-1ra was present at a much higher concentration than the other targets and is represented by a secondary vertical axis. Error bar = ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Stdev</th>
<th>pg/ml</th>
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<tbody>
<tr>
<td>VEGF</td>
<td>33.6</td>
<td>3.7</td>
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</tr>
<tr>
<td>FGF-2</td>
<td>5.4</td>
<td>2.0</td>
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</tr>
<tr>
<td>SCF</td>
<td>6.9</td>
<td>0.8</td>
<td></td>
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<tr>
<td>IL-1ra</td>
<td>1974.0</td>
<td>63.7</td>
<td></td>
</tr>
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</table>

**Table 1.** Cytokine/Chemokine concentration in cell lysates
Discussion

The umbilical cord serves as a conduit of nutrients for a fetus. Oxygenated nutrient rich blood is carried from the placenta to the fetus until the baby is born. This cord blood is rich in primitive stem cells, growth factors and immune cells that are naïve as they have to be compatible for baby and mother. Moreover, the use of allogeneic cord blood has been used for decades in greater than 500 patients with diseases such as Hurler’s syndrome, Duchenne muscular dystrophy and Krabbe’s disease. Most importantly it is safe.

**Liveyon regenerative medicine product** consist of cord blood that has been isolated in a sterile environment and vigorously tested. The product contains cells as demonstrated by AO/PI staining (Fig 1) as described on the label of the vial, it is positive for MSCs marker CD90 and negligible amounts of HLA-DR as defined by the ISCT (Fig 2). Most importantly, Liveyon regenerative medicine product contains growth factors and cytokines such as VEGF, SCF, FGF-2 and IL-1ra (Fig 3).

**Basic fibroblast growth factor** (FGF2) is a growth factor involved in many aspects of growth, development and healing. It is what maintains stem cells in growth, development and unchanged state. In addition, it plays an important part in the enhancement of bone and cartilage formation of. Thus, Liveyon regenerative medicine product which contains FGF-2 may aid in repair of degenerative joint disease.

**Interleukin 1 receptor antagonist** (IL-1ra) is a protein released by cells and is a natural inhibitor of the pro-inflammatory state. IL-1ra plays a key role in immunomodulation and inhibits production of the protein TNF-α which has been indicted in several autoimmune diseases. In addition, there is a direct correlation with increased TNF-α and disability, death and cognitive decline. Hence, the importance of IL-1ra release from cells and its ability to control the inflammatory response which occurs following many disease and trauma states. Liveyon regenerative medicine product contains IL-1ra in higher concentrations than other growth factors.

**Vascular endothelial cell growth factor** (VEGF) is a signaling protein produced by cells that stimulates Angiogenesis. VEGF is part of a system that helps restore oxygenation to tissues and cells when blood supply is inadequate. When cells are deficient in oxygen, VEGF release helps stimulate angiogenesis. This is of importance especially in tissues that are avascular such as chondrocytes in joints. In a study where VEGF was neutralized using an antibody, blood vessel was completely suppressed, along with impaired trabecular bone formation and expansion of hypertrophic chondrocyte zone. The recruitment and/or differentiation of chondroclasts and resorption of terminal chondrocytes decreased. Thus, the importance of VEGF in cartilage formation and repair.

**Stem cell factor** (SCF) may help direct HSCs to their stem cell niche and it plays an important role in HSC maintenance. In addition, it has been found to mediate cell survival, migration, and proliferation depending on the cell type. SCF plays a crucial role for such things as hematopoiesis, pigmentation, gut movement, and some aspects of the nervous system.

In summary, Liveyon regenerative medicine product contains many factors that may aid in host repair and healing. The data provided demonstrates that following thaw there are cells that fall into the ISCT criteria of MSCs as shown with flow cytometry. Liveyon regenerative medicine product contains growth factors that play a role in angiogenesis, growth and repair of cartilage and bone tissue which should serve well in orthopedic disease.
References


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