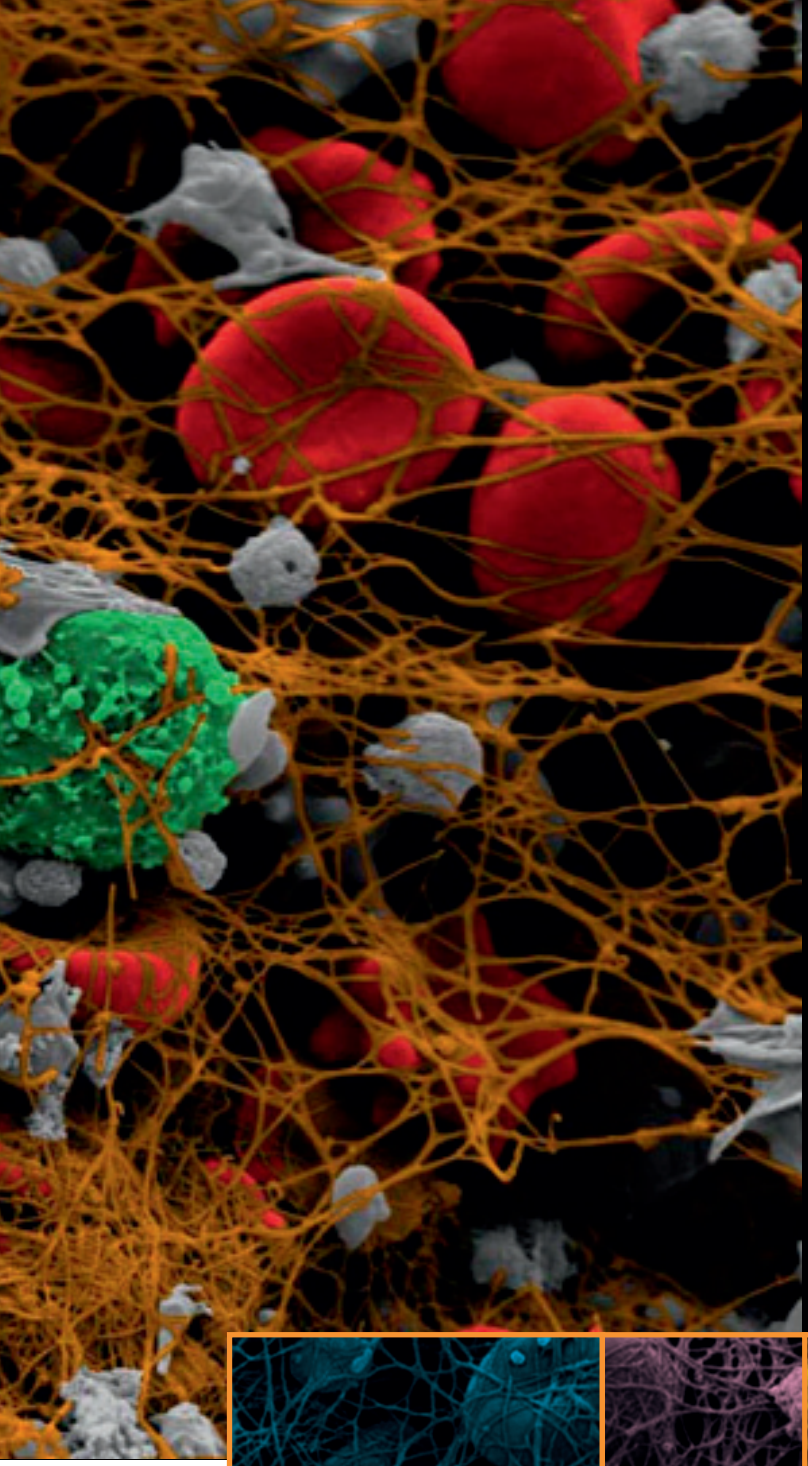
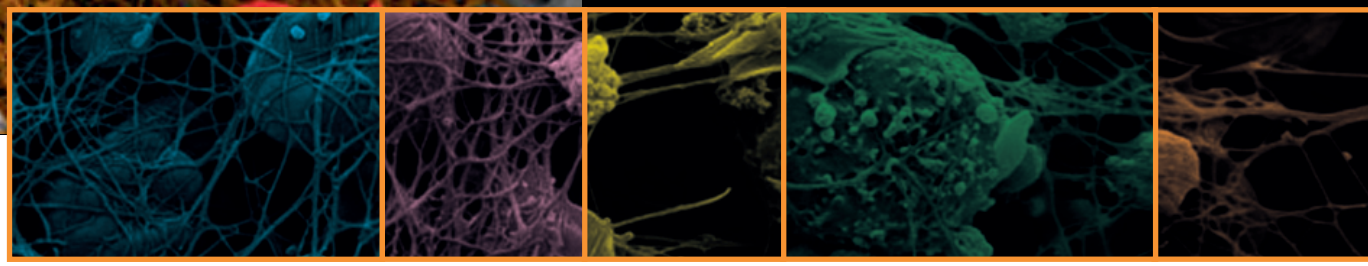


Lyset™



BOOST YOUR CELL CULTURE TODAY
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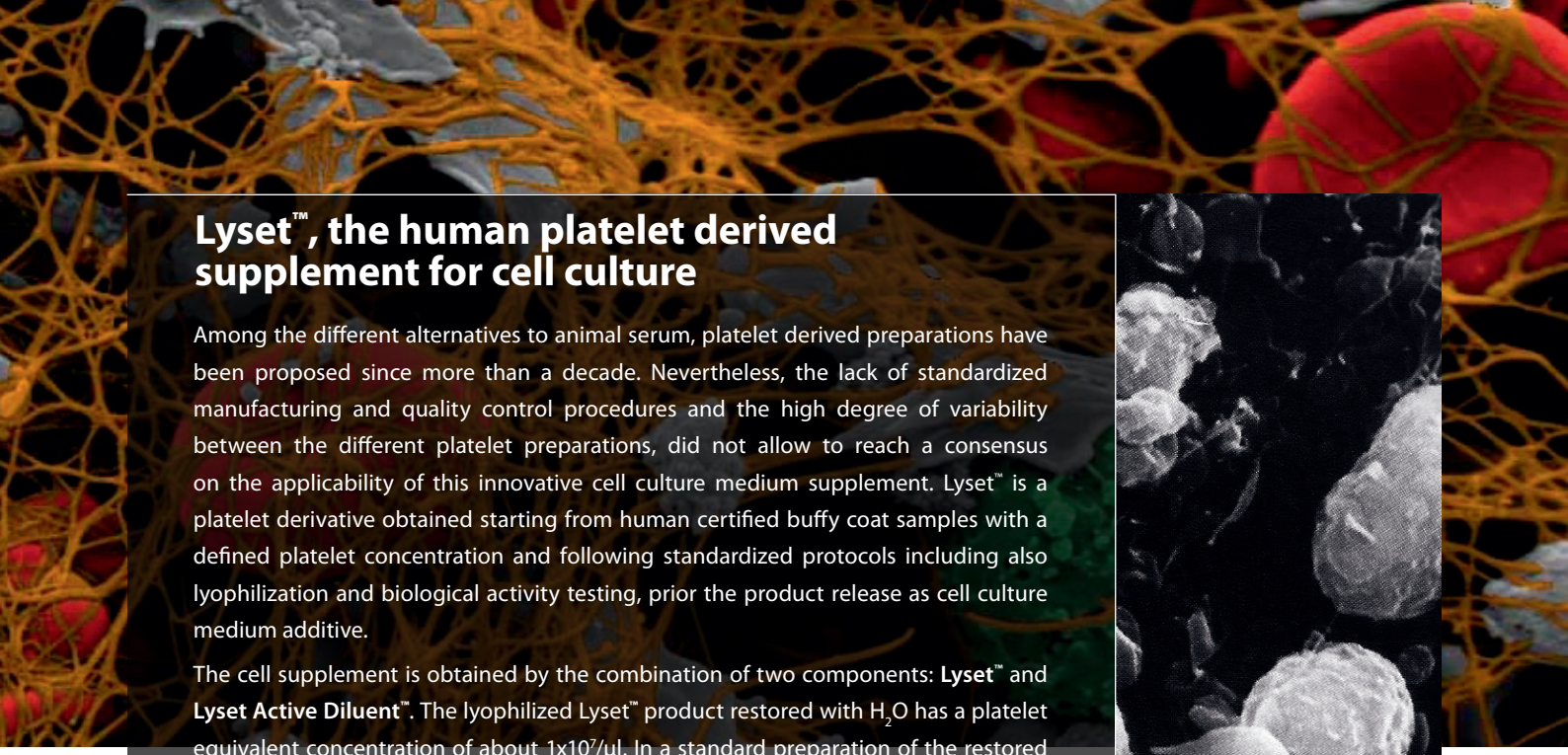


Lyset™, the human platelet derived supplement for cell culture

Among the different alternatives to animal serum, platelet derived preparations have been proposed since more than a decade. Nevertheless, the lack of standardized manufacturing and quality control procedures and the high degree of variability between the different platelet preparations, did not allow to reach a consensus on the applicability of this innovative cell culture medium supplement. Lyset™ is a platelet derivative obtained starting from human certified buffy coat samples with a defined platelet concentration and following standardized protocols including also lyophilization and biological activity testing, prior the product release as cell culture medium additive.

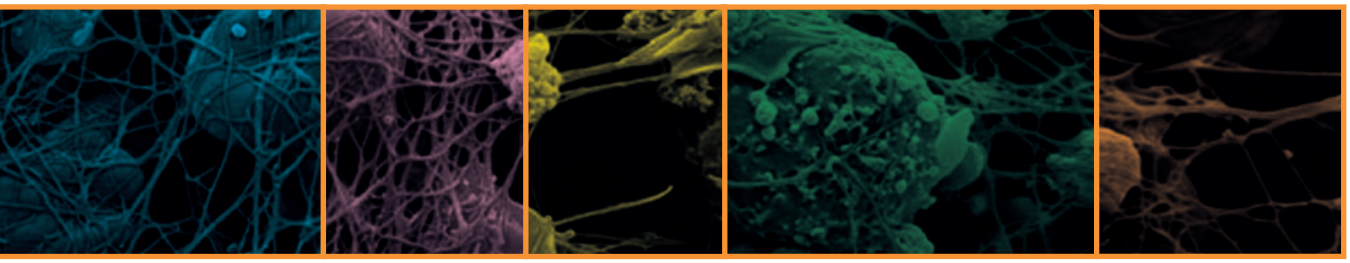
The cell supplement is obtained by the combination of two components: **Lyset™** and **Lyset Active Diluent™**. The lyophilized Lyset™ product restored with H_2O has a platelet equivalent concentration of about $1x10^7$/µl. In a standard preparation of the restored product the PDGF-BB and VEGF concentrations (quantified by Elisa assay) are at least 100 ng/ml for PDGF-BB and at least 2 ng/ml for VEGF. The lyophilized Lyset Active Diluent™ restored with H_2O has a platelet equivalent concentration of less than $5x10^4$/µl. The possibility of mixing the two supplement components in different percentages, allows the supplement customization and optimization for each cell type.

The Lyset™ is being utilized to replace animal and human serum such as Fetal Bovine Serum (FBS) in culture medium formulation for different types of cells. The Lyset™ was investigated in culture studies (cell growth, viability and product release) towards a number of target cells including fetal and adult stem cells, articular chondrocytes, osteoblasts, skin fibroblasts and other primary cells. In general the Lyset™ supplemented medium supported cell growth and maintained viabilities superior to fetal bovine serum supplemented medium.

Lyset™ contains only xeno-free components of human origin

Currently serum of animal origin and in particular FBS is the most commonly utilized cell culture medium additive for in vitro cell growth and differentiation. FBS represents a possible source of xenogeneic antigens and could be a vehicle for known and unknown pathogen transmission making cells cultured in the presence of FBS virtually unsafe for their potential use in regenerative medicine. In addition the use of animal serum limits the large-scale expansion of cells in cases where it is not possible to obtain cell proliferation in standard culture conditions (in the presence of FBS) due to the age of the donor.

To overcome these challenges Lyset™ has been developed as a culture supplement offering a xeno-free substitute of the animal serum in the cell culture medium.



In this brochure all pictures and specifications are purely indicative and may vary without notice.

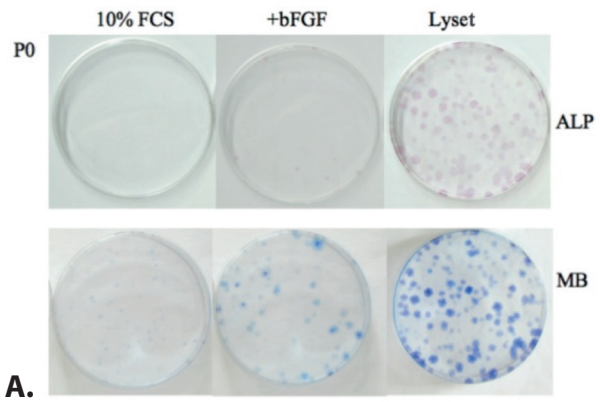
Lyset™ enables the selection and expansion of Mesenchymal Stem Cells from bone marrow

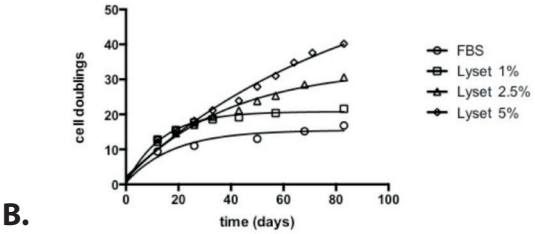
Lyset™ increases the number of clonogenic non hemopoietic human bone marrow derived cells and increases their number of doublings in culture maintaining the differentiation potential.

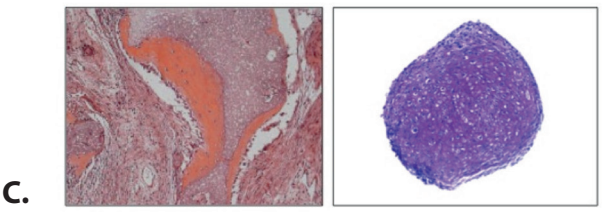
A. ***Lyset™ increases the number of clonogenic non hemopoietic human bone marrow derived cells (Mesenchymal Stem Cells - MSC).*** *Cultures were performed in parallel in medium supplemented with 5% Lyset™ and in medium supplemented with 10% FBS or 10% FBS+FGF-2. After 14 days from the initial plating the colonies were stained with Alkaline Phosphatase (ALP) and methylene blue and counted. A significant increase in the colony number was noticed in the culture supplemented with Lyset™.*

B. ***Lyset™ increases the number of doublings in cultures of human bone marrow derived Mesenchymal Stem Cells.*** *MSC population doublings were determined in cultures performed in medium supplemented with different Lyset™ concentrations or in culture medium supplemented with 10% FBS. Cells expanded in Lyset™ presented an enhancement of the proliferation rate, dependent on the Lyset™ concentration and underwent a higher number of population doublings.*

C. ***Mesenchymal Stem Cells expanded in the presence of Lyset™ maintain the differentiation potential.*** *Human bone marrow MSC selected and expanded with Lyset™ supplemented medium were seeded onto a porous ceramic biomaterial and implanted subcutaneously in immunocompromised mice (left panel). After 8 weeks from implantation, the histological analysis revealed abundant bone matrix formation (H&E staining). The in vitro chondrogenic stimulation in micromass cultures of the same cells expanded in the presence of Lyset™ (right panel) induced the deposition of an organized cartilagineous matrix (toluidine blue staining).*

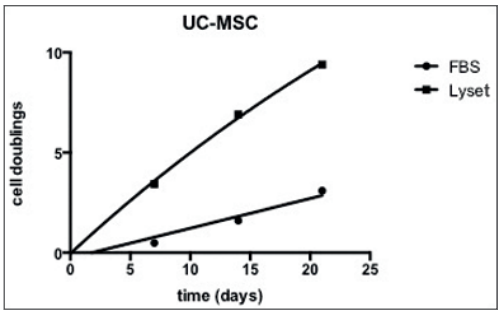


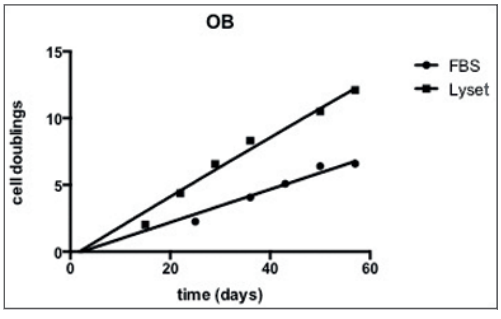


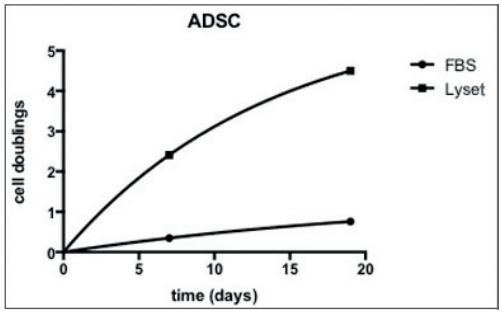


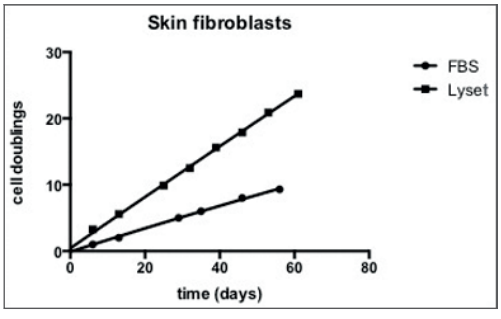
Lyset™ is particularly effective to increase the number of cells that can be derived from a tissue biopsy (primary cell cultures)

In general, the number of cell doublings that can be obtained starting from a primary cell culture, derived from a tissue biopsy, is higher maintaining the cells in a Lyset™ supplemented medium than in a fetal bovine serum supplemented medium.









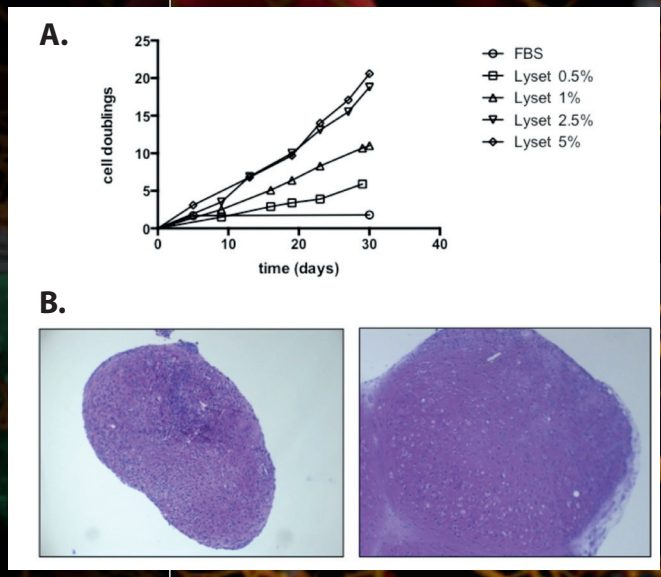
Cell doublings of human primary cells in culture. *Left panel: Osteoblasts; Middle panel Adipose Derived Stem Cells; Top right panel: Umbelical Cord Derived Mesenchymal Stem Cells; Bottom right panel: Skin Fibroblasts.*

Lyset™ allows the expansion in vitro of chondrocytes from articular cartilage biopsies of elderly subjects

Lyset™ allows the expansion in vitro of chondrocytes from articular cartilage biopsies of elderly subjects, when was not possible to obtain cell proliferation in standard culture conditions i.e. medium supplemented with 10% FBS. Human articular chondrocytes expanded in the presence of Lyset™ maintain the differentiation potential.

A. ***Lyset™ increases the number of doublings in cultures of human articular chondrocytes.*** *Articular chondrocytes expanded in* ***Lyset™*** *presented an enhancement of the proliferation rate, dependent on the* ***Lyset™*** *concentration and underwent a higher number of population doublings than cells cultured in standard culture medium (10% FBS). Interestingly supplementing the culture medium with Lyset™ allowed the expansion in vitro of chondrocytes from articular cartilage biopsies of elderly subject, when was not possible to obtain cell proliferation in medium supplemented with 10% FBS. In the example of the figure the cartilage biopsy was from a 77 year old man.*

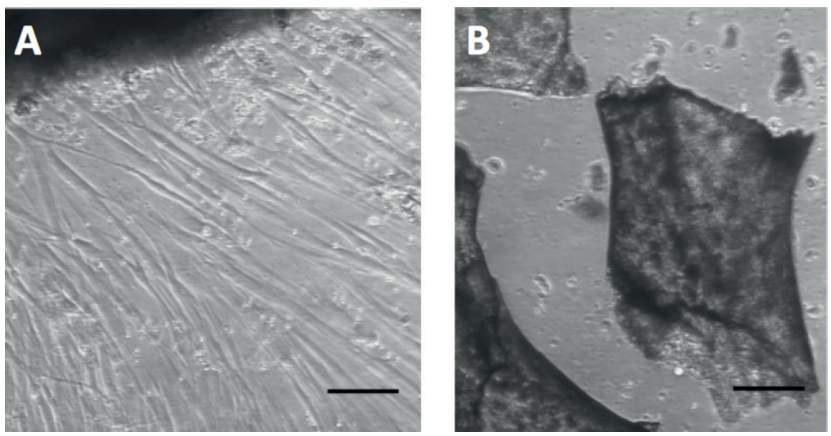
B. ***Human articular chondrocytes expanded in the presence of Lyset™ maintain the differentiation potential.*** *Human articular chondrocytes selected and expanded with Lyset™ supplemented medium were either induced toward a chondrogenic differentiation in micromass cultures (left panel) or subcutaneously implanted in immunocompromised mice (right panel). After 4 weeks from in vivo implantation, and 3 weeks of micromass culture, the histological analysis revealed the deposition of a well-organized cartilagineous metachromatic matrix positively stained with toluidine blue both in vitro and in vivo.*



Lyset™ promotes outgrowth of proliferating osteoblasts from bone chips

Human bone chip cultures were performed in complete medium either supplemented with Lyset™ or with FBS. After two weeks a massive outgrowth of cells from the bone chips is observed in the Lyset™ - containing cultures, but not in the FBS - containing cultures.

Release of cells from Lyset™- treated bone chips. *Bone chips were maintained in medium supplemented with Lyset™ (A panel) or FBS (B panel) for 12 days.*



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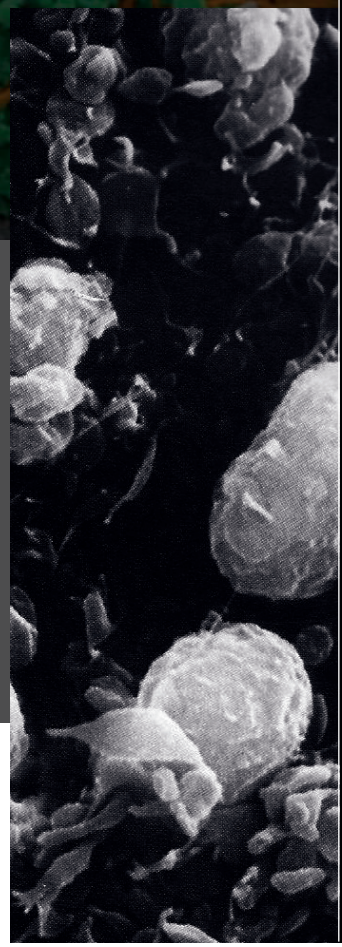
Lyset™ the platelet derived supplement for cell culture

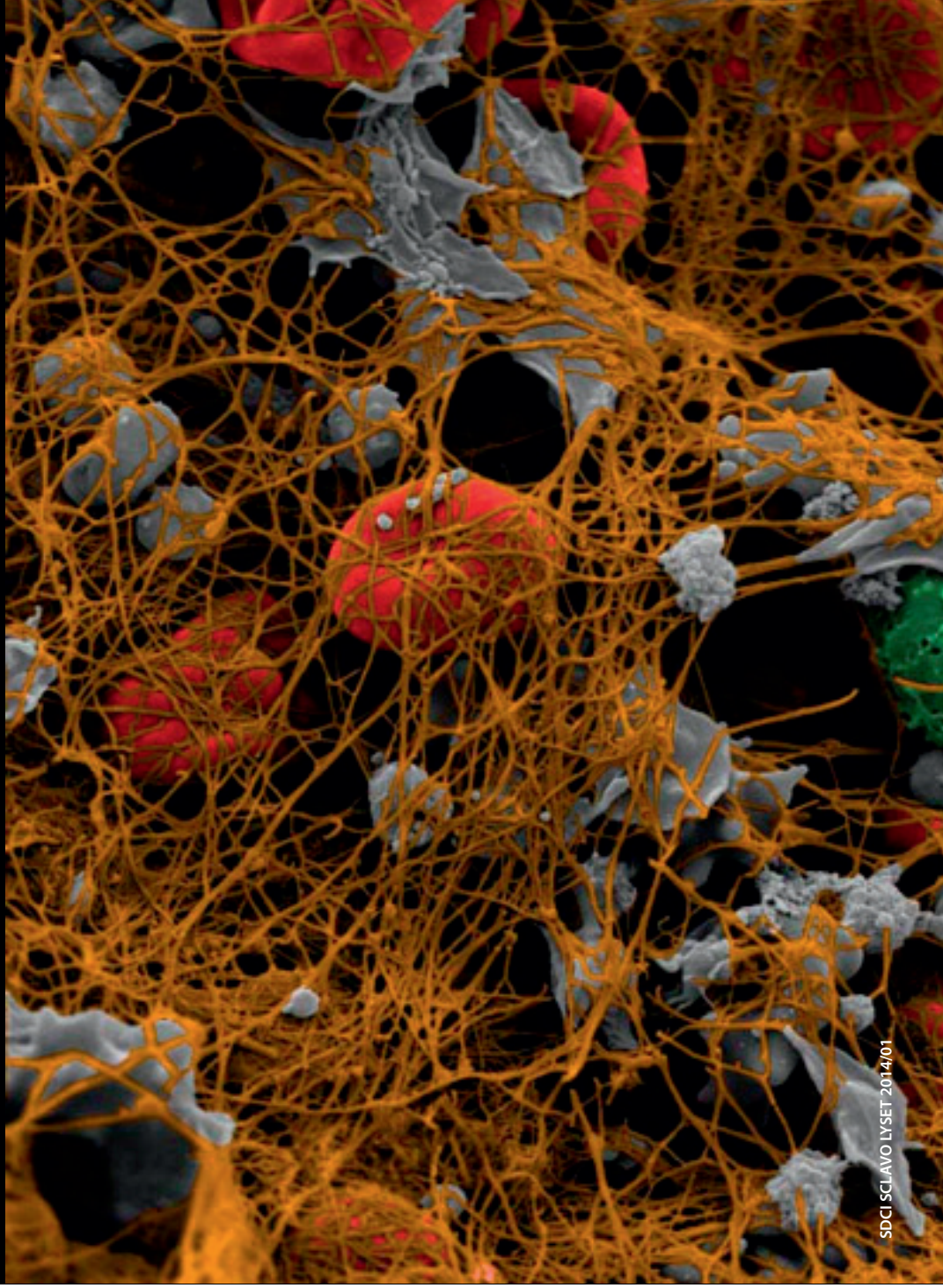
- an "off the shelf", fully standardized and lyophilized, human platelet derived cell culture supplement
- enterely of human origin for in vitro cell culture applications
- enables selection, expansion and differentiation of human primary cell cultures, such as adult and fetal mesenchymal stem cells (MSC), skin fibroblasts, articular chondrocytes, osteoblasts, adipose derived cells
- enhances cell proliferation maintaining the differentiation potential in cells otherwise difficult to expand in culture such as articular chondrocytes from elderly donors
- allows culture of cells in a xeno-free environment (as completely eliminates the need of Fetal Bovine Serum)
- the two component system is highly flexible and allows easy optimization of culture conditions for any cell type directly by the user



Blood platelets are reservoirs of growth factors and cytokines playing a major role in tissue repair and regeneration.

Platelets form a major proportion of both human and animal blood. The inner surface of blood vessels is lined with a thin layer of endothelial cells that, in normal hemostasis, acts to inhibit platelet activation. When a tissue damage occurs and the endothelial layer is injured, the platelets contact the subendothelial collagen and other extracellular matrix proteins are activated and clump together.

Activated platelets release a multitude of growth factors including platelet-derived growth factor, TGF beta, basic fibroblast growth factor, insulin like growth factor I, platelet-derived epidermal growth factor, vascular endothelial growth factor and other factors and active molecules which are present in the "optimal" concentration and relative percentage to play a significant role in tissue repair and regeneration.



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