“Serum-Free” medium for cultivation of mesenchymal stem cells

Stem cells have generated a great deal of interest in recent years due to their potential use in regenerative medicine (Fig. 1). Stem cells have been extensively characterized, but their therapeutic applications are still hampered by difficulties related to cell growth and storage, as well as by necessity of complying with strict regulations applied to cell-based clinical applications in humans. “Clinical grade” stem cells used for cell-based therapies must in fact exhibit special properties: they must be able to survive, reproduce and differentiate in artificial cell culture environments. More importantly, they must survive the process of freezing and of cryopreservation required for long term storage, as well as the subsequent thawing procedure that reactivates metabolic pathways required for *in vitro* expansion. Mesenchymal stem cells (MSCs) satisfy these strict requirements and thus represent unique candidates for cell-based therapeutic applications in various areas of modern regenerative medicine. MSCs are immature and pluripotent cells that can be expanded *in vitro*, and, following exposure to active biomolecules and transforming growth factors, differentiate into variety of specialized mature cells such as chondrocytes, osteocytes, adipocytes and cardiomyocytes (Fig. 1). Stem cells divide by a peculiar self-renewal mechanism that yields one daughter cell identical to the parent and one cell that is destined to differentiate. By this so-called asymmetric division, the populations maintains both staminality and a constant number of cells over time, thus providing a long lasting pool of stem cells that can also be found in adult organisms. In fact, stem cells represent a natural component of the human body and are distributed in various niches, such as the bone marrow, umbilical cord, liver, pancreas and skin. A novel source, adipose tissue, has been discovered recently and is now considered the richest niche of mesenchymal stem cells associated with the walls of small vessels. Novel strategies for the high-yield harvesting of stem cells therefore contemplate a relatively non-invasive procedure such as lipoaspiration to gain access to the source tissue, followed by enzymatic digestion of the lipoaspirate which yields a crude stromal vascular fraction (SVF). Subsequent isolation of mesenchymal stem cells relies on their morphological and phenotypic properties as well as their ability to adhere to plastic surfaces and therefore to expand *in vitro,* yielding the quantities required for applications in regenerative medicine. In fact, although they are a rich source of stem cells, lipoaspirates do not, at present, yield cell quantities sufficient for direct application in regenerative medicine.

Protocols for the *in vitro* expansion of clinical grade stem cells must comply with good manufacturing practice (GMP) regulations and may include only well-defined reagents of non-animal origin. In this regard, a major challenge lies in the development of culture media devoid of animal sera, a major component of traditional mammalian cultures, which provides the necessary nutrients for cell growth and exerts cyto-protective effects during cryopreservation. While they are a fundamental element for cell growth and storage, animal sera are of variable and ill-defined composition and are considered a potential source of contaminants such as viruses, toxins, or pyrogens, and hence must be eliminated from reagents involved in cell based therapies.

Aims

To develop GMP-compliant “serum-free” procedures for the isolation, *in vitro* expansion, differentiation and cryopreservation of adipose derived mesenchymal stem cells. The composition of the media involved must be entirely known, and devoid of animal components. Basal culture media must be further complemented with a set of carefully selected growth factors able to promote cell growth in the absence of animal sera. In addition, to produce high-yield vital stem cells able to grow and differentiate *in vitro* under specific conditions to generate, in the long term, a sufficiently large clinical-grade cell population to be employed in regenerative medicine.

Achievements

We designed and entirely defined a synthetic serum-free medium containing standard nutrients as well as growth factors. We demonstrated that vital and *in vitro* expandable mesenchymal stem cells can be quickly isolated under these restrictive conditions (Fig. 2). Hereafter, the same cells can be amplified in the serum-free medium, frozen for long term conservation and, after thawing, expanded at a later time. After having identified and protected the serum free formula (patent pending) we are entering in the second phase of

development, where the medium will be produced in pilot amounts of 50-100 l, ready to be tested by other laboratories outside SSCF.
Furthermore, the genomic stability (see specific project) and the epigenetic alterations of MSCs cultured *in vitro* are assessed in order to assure their suitability as therapeutics. We are currently establishing and validating analytical techniques that will be used to evaluate our long-term cultured cells.

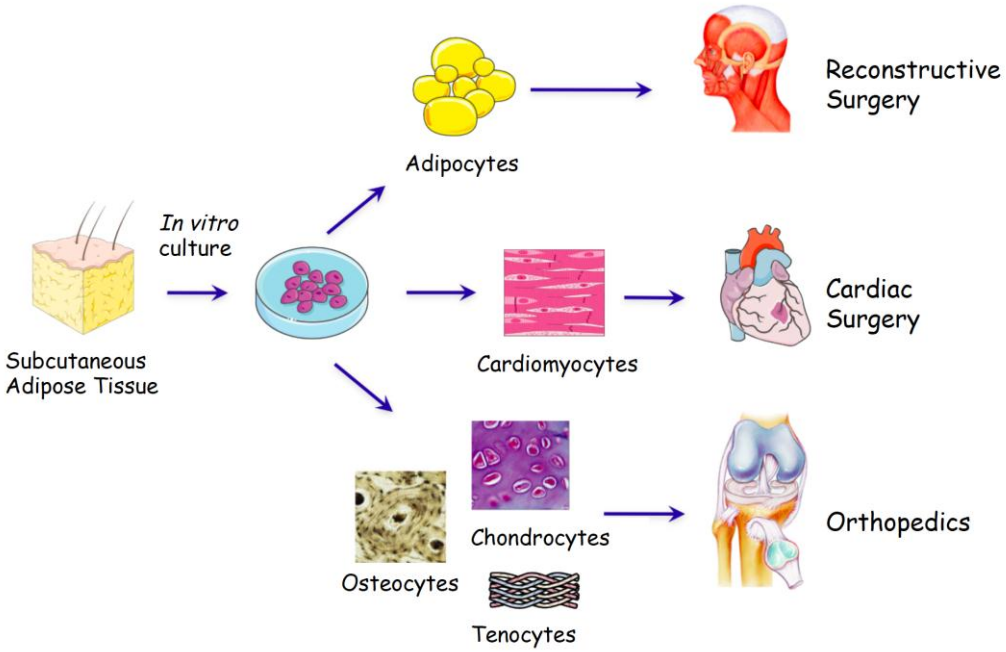

Fig. 1: mesenchymal stem cells (MSCs) can be extracted from adipose tissue and be cultivated *in vitro*. Under specific conditions, the cells can differentiate into specialized types (adipocytes, cardiomyocytes, osteocytes, chondrocytes and tenocytes). Thus, cultured MSCs can be used in several branches of regenerative medicine.

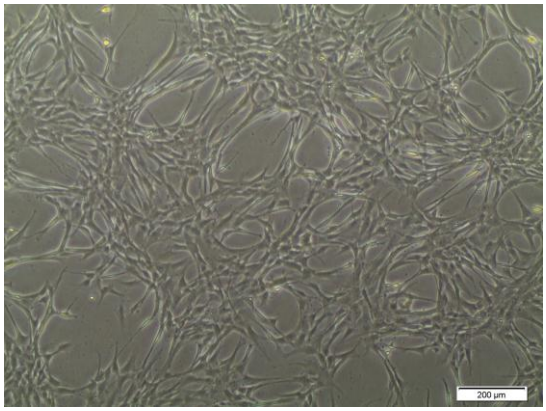

Fig. 2: mesenchymal stem cells cultivated *in vitro*.