USE OF HUMAN MESENCHYMAL STEM CELLS FROM AMNIOTIC FLUID AND CHORIONIC VILLI FOR BONE REGENERATIVE APPLICATION

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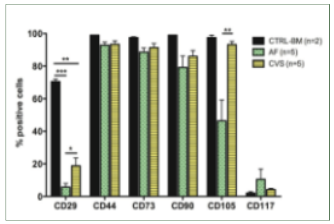
INTRODUCTION

Mesenchymal stem cells (MSCs) are an extraordinary tool for treating a variety of debilitating diseases such as chronic or degenerative diseases, GVHD and other numerous human conditions such as congenital malformations and traumatic injuries. In fact, MSCs have an high capacity to proliferate and are able to differentiate into many cell types, including skin, muscle, cardiac tissue, kidney, liver, bone, cartilage, neurons etc, hence expected to potentially treat tens of thousands of patients in a broad range of therapeutic applications. Amniotic fluid (AF) and chorionic villi (CV) are now emerging as one of the most useful source of MSCs for cellular therapy and tissue engineering because they contain far more pluripotent cells than other sources of MSCs such as the adult tissue, and also for their safety in terms of genetic stability and non tumorigenicity *in vivo*. In regenerative medicine, MSCs of fetal origin have been used in animal models for the reconstruction of diaphragmatic tendon, heart valves, bone grafts and trachea tracts, starting from very small quantities of initial cells. Here, we assessed the characteristics of AF and CV mesenchymal stem cells and tested their use for potential applications in bone tissue repair.

CHARACTERIZATION OF MSCS FROM AMNIOTIC FLUID (AF) AND CHORIONIC VILLI (CV)

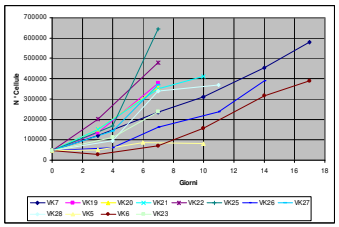
Starting from 3 mL of AF and approximately 5 mg of CV, the collected samples were analyzed for biological endpoints like: cell viability, proliferation rate, doubling times and immunophenotype by testing the expression of specific mesenchymal markers. The genome stability in culture was also verified by karyotype, wide-genome CGH-array and microsatellite analysis, and differentiation potential under specific culture conditions was investigated.

PHENOTYPICCARACTERIZATION



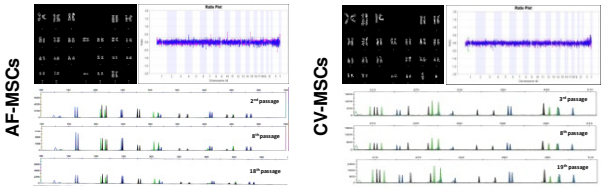
Protein analysis of AF and CV cell cultures show a mesenchymal stem cell profile comparable to that of control human bone marrow MSCs.

PROLIFERATIONCAPACITY



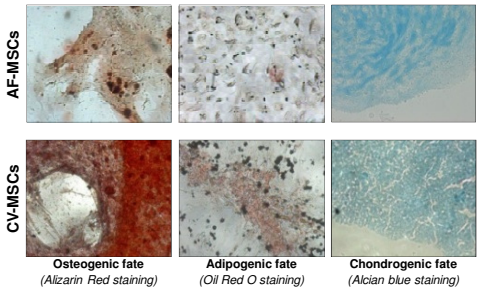
Five native AF and twelve CV samples were cultured and analyzed for cell viability and cell proliferation. Data show variable doubling times ranging from 45 to 494 hours.

GENOMICSTABILITY



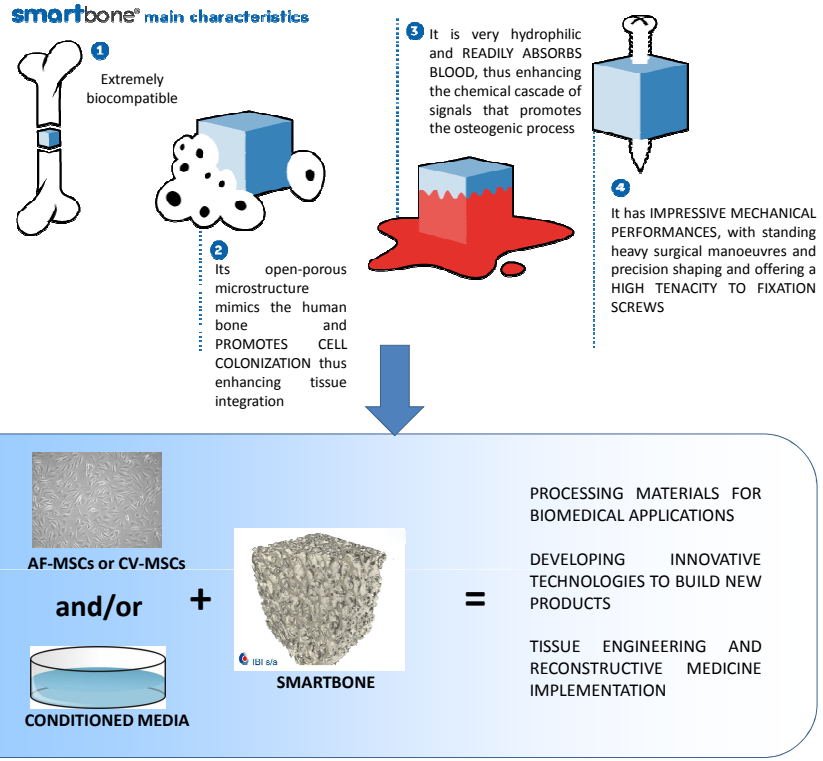
Analysis of genomic stability of cultured AF-MSCs and CV-MSCs reveal no alterations up to the 18th passage in culture, as demonstrated by karyotype analysis (top left panels), microsatellite analysis (top right panels) and CGH array (bottom panels). The results indicate that the *in vitro* culture do not induce any modification in genome stability.

DIFFERENTIATIONPOTENTIAL



Both AF-MSCs and CVS-MSCs have the ability to differentiate into osteoblasts, adipocytes and chondroblasts after stimulation with soluble factors *in vitro*, as demonstrated by histological staining specific for each tissue.

REGENERATION OF THE BONE



CONCLUSIONS

- Our findings indicate that it is possible to isolate and extensively expand AF-MSCs and CV-MSCs and that the in vitro culture does not interfere with the genomic stability.
- It is now possible to process biomaterials as SmartBone to develop and implement innovative technologies for reconstructive medicine.
- AF- and CV-MSC could be suitable for therapeutic purposes.
- The use of cell bank technology, on native samples, might represent a life-long available autologous cell source for perinatal and adult regenerative medicine.