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International Conference on
Stem Cells and Regenerative Medicine
&
2nd World Congress on
Pediatrics and Child care
November 06-07, 2019 | Tokyo, Japan

Keynote Forum

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STEM CELLS AND REGENERATIVE MEDICINE
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Regeneration of the immune system to fight leukemia at relapse post alloHSCT

The use of allogeneic hematopoietic stem cell transplantation in AML patients suffering from intermediate and high-risk disease was the real breakthrough in improvement of treatment results. AlloHSCT offers rebuilding of the normal hematopoiesis ruined by leukemia and chemotherapy as well as regeneration of the immune system which after transplantation has the immune system potential of healthy donor what also means that if exposed to leukemic blasts may identify them as alien. Therefore, allogeneic hematopoietic stem cell transplantation makes the immune response against the transplanted patient cells including the blasts possible. If the immune response is not effective enough the donor lymphocytes may be infused (DLI). This approach proved to be effective especially in chronic myelogenous leukemia and in some indolent lymphomas but not good enough in AML. Unfortunately, DLI associates with a considerably toxicity with acceleration of the graft vs host process as a main cause.

Having under our observation an ALL patient who relapsed after alloHSCT with leukemic infiltrations of the bones but not the marrow we injected the donor cells directly to the bone lesions with a positive effect. To exploit this approach further we started a project on the use of intra-bone route for injection of donor lymphocytes directly to the marrow cavity at relapse - thus providing the direct contact between the leukemic cells and the fresh lymphocytes from which those seeing blasts may be recruited.

Nine patients they relapsed after alloHSCT entered the experimental group having as counter-partners the patients they received at relapse standard therapy. The aim of the project was to evaluate the feasibility of the use of intra bone route and also to identify the cells which boosted in their potential by IB-DLI might be involved in anti-leukemic effect in other words in graft vs leukemia. The observation led to the following conclusions:

- The intra-bone route proved to be convenient and free from unwanted effects.
- The lymphocyte used for infusion were taken from the primary transplant material (stimulated) or obtained de novo from the blood with the use of leukophoresis (unstimulated), both cell populations except of the content of CD34+ cells did not differ especially the proportion of CD3+ cells was very similar.
- Local anesthesia and low molecular heparin secured that infusion procedure was undisturbed
- The patients which received IB-DLI enjoyed better 12- and 18-months survival as compared to those on standard therapy (77% vs 11%, p=0.006 and 55% vs 11%, p=0.035, respectively).

- The positive effect was seen rather in the patients having the leukemic cells invaded by CD8+ lymphocytes which proportion in the marrow cell population declined as leukemia vanished (3053±1036 vs 937±47 × 10E6 cells/L, $p<0.070$). The same was with the proportions of CD8+cells co-expressing PD-1 (1238±476 vs 255±73 × 10E6 cells/L, $p<0.060$).
- The key observation was associated with the analysis of the clonotype profiles (next generation sequencing) which showed that: (i) dominant clones identified in the recipients of IB-DLI were different from those seen in the lymphocytes prior to infusion (ii) the dominant clones rather persisted along the observation time even when the leukemia cells disappeared from the marrow, (iii) the profile of clonotypes in the marrow and in the blood was very similar in 32 out of 50 immunodominant clones what shows on the similarity between the immune system potential of the blood and marrow lymphocytes, however, the marrow lymphocyte had their local environment dependent distinctiveness.

In conclusion the IB-DLI (i) is feasibly, (ii) results with the improvement of the patients survival, (iii) is effective rather in those they have already responded to the leukemic cells with CD8+ cells but they had to be regenerated (PD-1 positivity of CD8+ cells) by providing fresh cells to achieve reversal of T-cell exhaustionand, finally, to exert clinically relevant activity.

Biography

Andrzej Lange graduated with a medical degree with distinction from the Medical School in Wroclaw, Poland is a professor in the Institute of Immunology and Exp Therapy of the Polish Academy of Sciences and a founder and head of the Lower Silesian Center of Cellular Transplantation in Wroclaw. His international experience started in 1973-1974 as a Leverhulme fellow in the Middlesex Hospital Medical School, London. He has been a visitor and lectured in several European and North American scientific institutions. Known from his activity in the field of bone marrow transplantation and regenerative medicine. Andrzej Lange was awarded several scientific distinctions and served to a number of National and European institutions were also active in co-editing journals. He is an author and co-author of 249 scientific papers, in peer-reviewed journals with a cumulative IF of 270 in the years 1995–2017.

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Jose Inzunza

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Genetic changes at chromosomal and DNA level during long term cultivation of hES cells

Human embryonic stem cells (hESCs) are important research tools in studies of the physiology of early tissue differentiation. In addition, these cells are regarded as a promising approach to generate transplantable cells for the treatment of several diseases and therefore offer an immense potential as a source of cells for regenerative medicine. However, the possible ability of these cells to produce tumors *in vivo* presents a major impediment for this achievement. hESCs can obtain growth advantages *in vitro* by acquired mutations. The mechanisms that may influence chromosome modification in hESCs are not well known. We have performed a comparative *in vitro* and *in vivo* study on hESC lines produced in our laboratory to see if there are changes also during *in vivo* growth. *In vivo* differentiated cells and *in vitro* cultured hESCs were analyzed by using first comparative genome hybridization (CGH) and second a high-resolution Affymetrix SNP 6.0 array revealing DNA copy number variations. We were able, for the first time; identify an aberrant X chromosome both *in vitro* and *in vivo* in one out of the 3 hESC line, we decteted an amplification of the whole X chromosome, possibly due to mosaicism of XY and XX cells. In the other hESC line, array results showed small amplifications and gains. The third hESC line was less altered but contained also a new gain verified by fluorescent in situ hybridization in a teratoma in 21% of the cells. These results indicate that mutations occur during the *in vivo* differentiation process as well as *in vitro*. The potential of precancerous mutations in *in-vivo* conditions is important to consider for safety measures and underlines the necessity to remove all pluripotent stem cells from the differentiated cell population that will be transplanted.

Biography

Jose Inzunza is an associate professor and senior researcher at the Department of Biosciences and Nutrition, Karolinska Institutet (KI). He received his doctorate in obstetrics and gynecology at Karolinska University Hospital, KI. With a specialization in cytogenetics, he worked on his doctoral work with a project in clinical application of preimplantation genetic diagnosis (PGD). He has also been involved in the development and implementation of the laboratory for derivation and differentiation of human embryonic stem cell research at KI. This was the first bank of human embryo stem cells in Sweden and Scandinavia. Jose has also worked with cellular re-programming. Today, Jose's line of research is in stem cells and tumorogenesis and genetic stability of these cells during the differentiation process.

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Fabrication and characterization of electrospun PCL nanofibrous scaffolds for tissue engineering: Biomechanics and cells behavior

Statement of the Problem: Tissue engineering is a promising solution for the problem of organ or tissue shortage. A main requirement is the use of biologically functional scaffolds to deliver cells to the implant site and/or provide a structure for cell attachment to regenerate lost or damaged extracellular matrix (ECM). The natural ECM is structured in the nanoscale range, a characteristic that should be incorporated into scaffold design for tissue engineering. Scaffolds produced by the electrospinning process have several unique advantages. In this research, we survey the potential of poly(ε-caprolactone) (PCL) for the synthesis of electrospun nanofibrous scaffolds and investigate their biomechanics and cell's interaction for successful tissue engineering applications.

Methodology & Theoretical Orientation: PCL pellets were dissolved in acetic acid (20% wt.). Electrospinning was implemented to manufacture the microporous nanofibrous scaffolds. Morphological characterization was observed by SEM. Mechanical tensile testing and *in vitro* degradation of the scaffolds were also performed. The MTT assay was used to determine viability of hCMEC/D3 cell line following exposure to electrospun PCL scaffolds surface.

Findings: Results showed a scaffold morphology consisting of parallel, aligned and homogeneous PCL microfibers with diameter 1.16 ± 0.45 um, pore size 17.7 ± 5.37 um (Figure 1) and measured elastic modulus 18.3 ± 0.23 MPa, in the fibers direction. Gravimetric weight loss of the PCL scaffolds immersed to PBS (37°C) was measured weekly over 15 weeks (4-10% weight loss). Capability of cell infiltration verified by MTT assay where cytotoxicity was not observed, exhibiting high cell viability (85.64 ± 3.12%).

Conclusion & Significance: Utilizing the electrospinning process we were able to produce laminate micro fibrous PCL scaffolds. Their structural organization and biomechanics mimic natural tissue ECM structure and bio-functionality, as well as they are capable of hosting cells. This material (PCL) appears to be a promising candidate for tissue engineering applications.

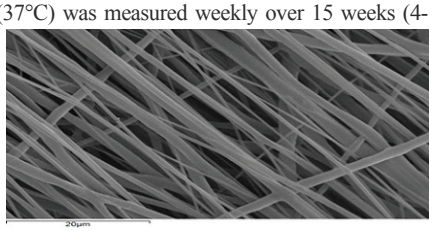

Figure 1. SEM image of PCL electrospun nanofibrous membrane

Biography

Dimosthenis Mavrilas is a professor of Biomedical Engineering in the Laboratory of Biomechanics and Biomedical Engineering, Department of Mechanical Engineering & Aer/tics, University of Patras, Greece. He is an expert in biomechanics of biomaterials and biomedical engineering of cardiovascular system. Last decade his research targets in tissue engineering, producing scaffolds either of biological origin (decellularized animal tissues) or from synthetic polymers. His research team achieved the production of either random or parallel fibrous orientation of synthetic biodegradable polymeric nanofibers, capable for the structure of multi laminate biomimetic scaffolds, suitable especially for cardiovascular tissue engineering.

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