ImmunoTools *special* Award 2014

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Bio-fabrication of Artificial Tissues for Tissue Engineering

Tissue engineering is a very attractive branch of modern medicine. In fact, it represent not only a promising vehicle to face the “regenerative medicine” requests, but it is also a very sophisticate tool for the *in vitro* studies of complex biological systems such as tissues development and regeneration. A great step forward in tissue engineering advancement is represented by the improvement of biomimetic materials that are able to active interact with cells in order to provide microenvironments suitable for the clinical application and the basic theory progress.

During my PhD, I developed some innovative materials suitable for tissue engineering applications; in particular, I focused on the surface modification of medical (grade 2) titanium for hard tissues (bone) regeneration and a thermo-reversible methylcellulose-derived hydrogel for soft tissue (skin) regeneration. The surface modifications have been developed in order to improve the adhesion and to promote the proliferation of the tissue-specific cells (osteoblasts for bone and fibroblasts for skin) with the purpose to improve the repair and the regeneration of the damaged tissue where the materials could be implanted. As the surface modifications are innovative, from a biological point of view, *in vitro* cytocompatibility is the first evaluation necessary to validate the materials. In order to do that, cells are seeded directly onto the materials surface and some biological parameters are evaluated. First of all, materials surface modifications must not inhibit cells adhesion; therefore, the use of specific markers related to adhesion protein such as collagen, integrins and hyaluronic acid could be an important proof to demonstrate that cells are attached and are successfully promoted to produce their natural extracellular matrix (ECM). So, cells positive response to specific antibody such as CD54, 47, 41, 36, 29 and 18 represent an excellent tool to answer to this first question. Then, once cells are attached, they must also be able to proliferate and reproduce a complex structure

suitable to develop a biochemical microenvironment for the cell-to-cell cross talk. Annexin 5 and CD95 are specific markers related with apoptosis; in fact, Annexin 5 is representative for the phosphatidylserine nuclear translocation while CD95 is related with the caspase 8 cascade activation. Thus, by using these specific markers it is possible to understand if cells seeded onto materials surface are undergoing into apoptosis or not.

Once the *in vitro* cytocompatibility has been verified, the successive step is to *in vivo* assay the material composite biocompatibility. The technique that I normally use is to subcutaneously implant the materials into recipient wild-type mice and to evaluate after an adequate period (I) the integration to the naïve tissue and (II) the eventual immunological reaction related with the implant. The point (I) is crucial to verify that the material modifications are effective to improve the compatibility with the naïve tissue. Therefore, it is necessary to verify that the implanted materials are successfully colonized by the murine cells that must be able to adhere, spread, proliferate and recreate a microenvironment suitable for the formation of new tissue. Therefore markers specific for cell-to-cell and cells-surrounding tissue such as CD44 and 11b could help to verify this hypothesis. Moreover it will be very interesting to investigate the expression of CD117 that bind cells expressing c-kit, one of the most important gene related with the adult cells stemness potency. The point (II) is crucial to exclude a severe inflammatory response related to the materials implant. Therefore the use of specific markers such as CD3e and NK-cells could help to identify the presence of an inflammatory process.

The final step is the validation of the regenerative potency of the cells-materials composites; in this case it is necessary to recreate an artificial defect to verify if the composites are able to promote the regeneration of the tissue.