# ImmunoTools *special* Award 2014

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## Implantation of Mesenchymal Stem Cells in surgical meshes for prevention of inflammatory-mediated responses

Surgical meshes are widely used in a variety of medical procedures, such as hernias and pelvic floor defects. The non-absorbable meshes are among the most frequently implanted biomaterial, however, chronic inflammation is commonly observed after surgical interventions. Here we hypothesize that implanting of mesenchymal stem cells (MSCs) associated to surgical meshes may have beneficial effects on the wound healing, closure properties and a decreased risk of inflammatory reactions after surgery.

It has been extensively demonstrated that MSCs are a useful clinical tool for treatment of many diseases and have been recently used in combination with sutures to improve the strength of adhesion-free colonic anastomoses [1]. Similar biosutures have been developed using bone-marrow MSCs demonstrating decreased intra-abdominal adhesions [2]. For clinical applications, modified surgical meshes could be somehow considered as a scaffold to hold the cells, providing a biomechanical support to avoid the spreading of MSCs.

The aim of the present work is to test the anti-inflammatory properties of MSC-coated surgical meshes in a mouse peritonitis model. In this work, murine stem cells will be characterized by flow cytometry using a combination of stemness markers (CD34, CD44, CD90, CD29...). These markers will be analysed in those MSCs adhered to the meshes. Additionally, viability and apoptosis will be analysed in adhered cells using the ImmunoTools reagent Annexin-V FITC. Surgical interventions will be performed on ICR mice and meshes will be intraperitoneally implantated. Peritonitis will be induced by thioglycolate injections. The thioglycollate-induced peritonitis in mice is used as a model to study for potential anti-inflammatory action of investigated test compounds [3]. The thioglicolate-mediated inflammation will be monitored at 72 h by intraperitoneal lavage. The analysis of different cell subsets (lymphocytes, NK

cells, macrophages or neutrophils) will be monitored by flow cytometry by using ImmunoTools antibodies against TCR, CD3, CD4, CD8, NK-cells or GR1...). Moreover, activation markers for in vivo monitoring cell activation will be analysed by multiparametric flow cytometry using ImmunoTools antibodies against CD25, CD29, CD134 CD62L and others.

Finally, for a future clinical application of these MSCs-coated meshes we aim to analyse different cytokines by human ELISA from ImmunoTools.

These ELISA tests will be performed in serum samples from patients submitted to surgical interventions using surgical meshes. The TH1 response (monitored by IL-12p40 and TNFalpha) and TH2 response (monitored using IL-4 and IL-6) will be quantified by ELISA kits from ImmunoTools.

References

1. Pascual I, Fernandez de MG, Garcia AM, Garcia-Olmo D. Biosutures improve healing of experimental weak colonic anastomoses. Int J Colorectal Dis. 2010;25:1447-1451.
2. Dolce CJ, Stefanidis D, Keller JE et al. Pushing the envelope in biomaterial research: initial results of prosthetic coating with stem cells in a rat model. Surg Endosc. 2010;24:2687-2693.
3. Fakhrudin N, Waltenberger B, Cabaravdic M, Atanasov AG, Malainer C, Schachner D, Heiss EH, Liu R, Noha SM, Grzywacz AM, Mihaly-Bison J, Awad EM, Schuster D, Breuss JM, Rollinger JM, Bochkov V, Stuppner H, Dirsch VM. Identification of plumericin as a potent new scaffold inhibitor of the NF-kB pathway with anti-inflammatory activity in vitro and in vivo. Br J Pharmacol. 2013 Dec 16.

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includes 25 reagents

FITC - conjugated Annexin V,

human IL-4 ELISA-set for 96 wells, human TNFa ELISA-set for 96 wells (each 3 reagents),

FITC - conjugated anti-mouse CD3e, CD11b, CD19, CD29, CD44, CD90, CD117, CD134, g/d TCR,

PE - conjugated anti-mouse CD4, CD81, Gr-1, a/b TCR,,

APC - conjugated anti-mouse CD3e, CD8a, CD49d, CD62L, NK-cells

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