

EFFECT OF MESENCHIMAL STEM CELLS FILM ON THE INFLAMMATORY RESPONSE AROUND TWO DIFFERENT MESH MATERIALS

Hypothesis / aims of study

Implant materials are increasingly being used in an effort to reduce recurrence after prolapse repair with native tissues. Constructs can be classified according to the source in synthetic, bio-derived and hybrid. So far an ideal implant material for pelvic surgery has not been identified. Most side effects related to permanent synthetic implant materials result from shrinking, erosion or deformation of the mesh. Biologic materials have been introduced as an alternative to synthetic ones because of their biocompatibility. Bio-grafts however, have potential limitations, including their limited supply, low tissue strength and unpredictable behaviour on medium and long-term.

Mesenchymal Stem Cells (MSCs) have been widely used in tissue engineering to repair and regenerate damaged tissue. MSCs are relatively easy to culture, to process and to store. MSCs need a scaffold to grow, and the differentiation process is driven by the microenvironment at the site of implant. In vivo the presence of a layer of MSCs surrounding the surface of the implant can improve its biocompatibility at the interface with host native tissue. The aim of this study is to compare inflammatory response through blood samples after implantation in a rat model of two different implant materials that are either surrounded or not by rat-derived MSC (rMSC).

Study design, materials and methods

rMSC were isolated from 10-weeks old Sprague-Dawley rat (Harlan, Udine, Italy) bone marrow and cultured in complete alpha-MEM medium, i.e. with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 250 µg/ml fungizone (Euroclone, Pero, Italy), supplemented with 20% heat inactivated Fetal Bovine Serum (Hyclone, Logan, UT, USA) in a humidified atmosphere at 37°C and 5% CO₂ in air.

rMSC (passage 3-4) were harvested by trypsinisation (0.05% trypsin/EDTA solution for 5 min at 37°C) and counted. In the meanwhile the materials of interest, Surgisis (Cook, Italy) and Pelvitex (Bard, Italy) - selected by previous in vitro experiment - were cut into 2 cm by 2 cm squares and placed in 35-mm cell culture plates under sterile conditions. 25x10⁶ cells were seeded in 0.5 ml alpha-MEM complete medium with 10% heat inactivated Fetal Bovine Serum onto Surgisis or Pelvitex and maintained in a humidified atmosphere at 37°C and 5% CO₂ in air. Cells were allowed to adhere to the mesh and after 2 hours 1.5 ml of fresh medium was added. After 3-4 days the materials were implanted

Forty-eight Wistar female rats were divided into 4 groups of 12 rats each further referred according the material implanted and the time point at sacrifice (7 and 90 days): Pelvitex without stem cells (PN), Pelvitex with stem cells (PS), Surgisis without stem cells (SN) and Surgisis with stem cells (SS).

The rats were operated under general anaesthesia and sterile conditions. After raising skin flaps, on the right side of abdominal wall the mesh was placed and fixed "tension free" at the corners by a 4/0 polyglecaprone (Monocryl, Ethicon) suture. Euthanasia was performed at the given time points.

Each rat served as its own control by collecting two blood samples from the tale-vein: one before surgery and one before sacrifice. Blood samples were analyzed for a full emocromocitometric examination including Blood Red Cells (BRC), Blood White Cells (BWC), Neutrophils (N), Lymphocytes (L), Macrophages (M), Eosinophils (E), Basophils (B), Platelets (PLT), Hemoglobin (Hb) and Hematocrit (Ht) counts.

Results

At 7-day time point, rats that were implanted with rMSC surrounded Pelvitex displayed higher counts of BWC (p value: 0,015) and lower counts of E (p value 0,028) compared to the ones implanted with Pelvitex without rMSC.

At 90-day time point, rats that were implanted with rMSC surrounded Pelvitex displayed higher counts of BRC (p value: < 0,0001), BWC (p value: 0,008), PLT (p value: 0,011), Hb (p value: 0,0005), Ht (p value: < 0,0001), L (p value: 0,017), B (p value: 0,002) and lower counts of N (p value: 0,003) and E (p value: 0,025) when compared to the ones implanted with Pelvitex without rMSC.

At 7-day time point, rats that were implanted with rMSC surrounded Surgisis displayed higher counts of N (p value: 0,002), M (p value: 0,026) and lower counts of L (p value: 0,0004) compared to the ones implanted with Surgisis without rMSC.

At 90-day time point, rats that were implanted with rMSC surrounded Surgisis displayed higher counts of M (p value: 0,004) and PLT (p value: 0,0003) compared to the ones implanted with Surgisis without rMSC.

Interpretation of results

After implantation of rMSC surrounded meshes, the early and late inflammatory response seems to grow in both the implants of interest.

Pelvitex is a low-weight and highly porous polypropylene monofilament mesh protected by a hydrophilic film. Surgisis is biodegradable small intestinal submucosa derived mesh-construct. In both biological and synthetic meshes the use of rMSC film seems to modify inflammatory response.

In a synthetic material enhanced inflammatory response can lead to important side effects such as erosion, fibrosis and exposure of the mesh. Biodegradable constructs have a significant lower risk of side effects but their efficacy is still uncertain. An increased inflammatory response can lead to a higher collagen deposition at the interface of the mesh. This phenomenon is advisable when mechanical resistance is required at the site of implantation. A denser and thicker collagen deposition could improve the tensile strength of biological tissues and this would have important influences on clinical results with the use of this product.

Concluding message

rMSC surrounded mesh-constructs increase the systemic inflammatory response in vivo. The consequences could be relevant in clinics. But more has to be studied to correlate inflammatory response to collagen characteristics and biomechanical properties.

References

1. International Congress Series 1279 (2005) 387–397
2. Int Urogynecol J (2006) 17: S16–S25

<i>Specify source of funding or grant</i>	None
<i>Is this a clinical trial?</i>	No
<i>What were the subjects in the study?</i>	ANIMAL
<i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i>	Yes
<i>Name of ethics committee</i>	Ethics Committee of University of Milano-Bicocca