Evaluation of a Polyamide-Gelatin Mesh seeded with Human Endometrial Mesenchymal Stem Cells for the repair of Pelvic Organ Prolapse

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Introduction:

Pelvic Organ Prolapse is defined as the descent or herniation of one or more of the pelvic structures into the vagina and is largely brought on after childbirth, affecting 44% of parous compared with around 6% of non-parous women. The treatment of Pelvic Organ Prolapse includes conservative surgical treatment and/or implantation of a synthetic or a biological mesh. However, the long-term outcome of synthetic mesh surgery, largely based on the use of polypropylene type meshes, has been unsatisfactory. In particular, this has been due to surgical failure and post-surgical complications, including lack of tissue integration and erosion of adjacent tissues.

The aim of this study has been to examine a tissue engineering approach to improve the *in vivo* performance of a new mesh implant. The performance of a synthetic polyamide/gelatin composite mesh implant used to deliver human endometrial mesenchymal stem cells (eMSC) in a rat model of wound repair was examined.

Methods:

Human endometrial mesenchymal stem cells (eMSC) were isolated from hysterectomy tissue using W5C5-labelled magnetic beads (Masuda et al., Cell Transplant. 2012; ePub) Cells were expanded in culture for up to 6 passages, where they retain multipotency (Rajaraman et al., Tissue Eng. C Meth. 2012; ePub). Scaffolds for implant were warp knitted from 100 μm polyamide monofilament, heat set for 30 sec at 200 °C, giving 85 g/m^2 and pores of ~1.3 mm. Mesh was coated with 12% porcine 300 Bloom gelatin and crosslinked by 0.025% glutaraldehyde. Samples were washed, dried and sterilized with 25 kGy γ-irradiation, giving a final 145 g/m^2. eMSC were fluorescently labeled with Vybrant® DiO and then seeded onto scaffold, 25 x 10 mm, with 250,000 cells/sample. Samples were implanted subcutaneously in the dorsal region of immuno-compromised rats for 7, 14, 30, 60 and 90 days (n=8/time point). Controls received mesh alone.

Flow cytometry was used to detect eMSC after explant. Immunohistochemical assessment of foreign body reaction and tissue integration was performed using antibodies to collagen types I and III, to CD31, CD45 and CD68 and to M1(pro-inflammatory) and M2 (anti-inflammatory) macrophage markers. Samples were also examined by quantitative histochemical assays and by collagen birefringence. New collagen formation was examined by quantitative analysis of the ratio of collagen type III to type I per dry weigh tissue using SDS-electrophoresis with delayed reduction and hydroxyproline analysis. Tensile testing was performed using an Instron Tensile tester.

Results:

The polyamide/gelatin mesh was readily seeded with eMSC and in vitro culture showed that the mesh was not cytotoxic and that the cells proliferated readily (Fig. 1).

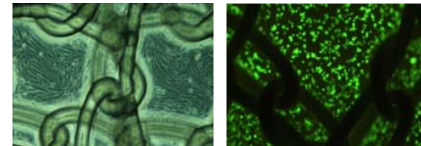

Fig. 1: eMSC cultured on gelatin-coated PA scaffold

Implanted meshes were well tolerated with no erosions. eMSC were detected on explants up to 14 days post-implant. Meshes with eMSC attracted significantly fewer leukocytes at 7 days ($p < 0.05$) and fewer macrophages after 30 days than the control samples. There were differences in the distribution of M1 and M2 macrophages over time (Fig. 2). Meshes with eMSC had significantly more neovascularisation at 7 days ($p<0.05$) (Fig. 3)

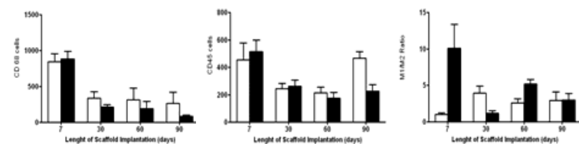

Fig. 2: Cell distributions in explants of mesh with eMSC ■ and without cells □. CD 68, CD 45 and M1/M2 ratio.

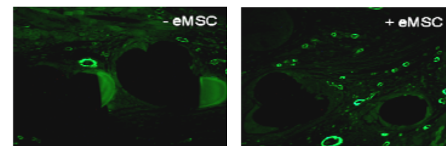

Fig. 3: Enhanced neovascularisation when eMSC were present.

New collagen production was observed at all time points in meshes with and without eMSC. By 90 days, there were differences in the mechanical properties of the cell seeded and control mesh, as well as in collagen type III/I ratio (Fig. 4) and collagen organisation by birefringence.

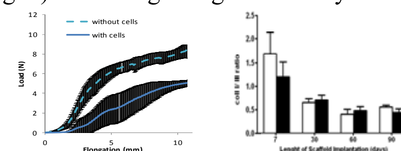

Fig. 4: Changes in mechanical properties and in collagen ratios.

Conclusions:

This tissue engineering approach significantly reduces the number of inflammatory cells around implanted mesh, promoted neovascularisation and improved the distensibilty of the mesh in the long term. This suggests that eMSC exert an anti-inflammatory effect and promote wound repair. Thus, eMSC delivered on polyamide /gelatin composite mesh might be an alternative option for future treatment of Pelvic Organ Prolapse.

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