Localized Recruitment of Multipotent Mesenchymal Stem Cells by Biomaterial Implants

Robert H Hansen III, Jinhui Shen, and Liping Tang
Department of Bioengineering, the University of Texas at Arlington, Arlington, TX 76010.

Statement of Purpose: Almost all biomaterial implants trigger different extent of inflammatory and fibrotic reactions. To determine the potential relationship between these two most common biomaterial-associated tissue responses, a series of studies were carried out to characterize the function and types of fibrotic cells surrounding biomaterial implants. Surpringly, we identified a unique type of undifferentiated multipotent stem cells which were recruited in abundance at the implantation site. This work was aimed at assessing the mechanisms of biomaterial-mediated stem cell recruitment and the multipotent properties of these recruited stem cells.

Methods. To mimic biomaterial-mediated inflammatory responses, two polyethylene terephthalate disks (Φ=1.2cm) were implanted into the peritoneal cavity of Balb/c mice as described earlier [1]. At different time points after the surgery (0h, 6h, 12h, 18h, 1d, 2d, 4d, 7d, 10d, 14d), cells recruited to the peritonea were recovered by lavage with 4mL of DMEM media. After centrifugation, the lavage cells were isolated and the total cell numbers were counted. The cells were used for cell proliferation study, FACS array analyses, immunocyto-chemistry assay and cell differentiation studies. For cell proliferation studies, the cells were cultured with DMEM supplemented with 15% fetal bovine serum (FBS) and penicillin (100unit/ml) and streptomycin (100μg/ml).

Results: Unlike cells present in the undisturbed peritoneum (resident macrophages and mesothelial cells), the adherent lavage cells from implant bearing animals had a fibroblast-like morphology (Figure 1A) and proliferated for periods of over 6 months. The growth kinetics of these fibroblast-like cells fit an exponential curve. On the other hand, almost no replicative cells were recovered from peritonea of control (unoperated) and very few were recovered from animals receiving sham surgery (Figure 1B). Using both flow cytometry and immunocytochemistry analyses, we determined that these cells stained positive for CD44, CD54, SH2, CD106, Collagen type I, SCF, CD11b and SSEA4; and negative for CD34, CD45.

To test the plasticity of these cells, further studies were done to determine the osteogenic, adipogenic, and neurogenic properties of these cells. Indeed, following an osteoblast differentiation protocol with added BMP-2 [2], the peritoneal lavage cells adopted an osteocytic phenotype, forming ossicle-like structures and stained positive for calcium with Alizarin Red S (Figure 1C). These peritoneal lavage cells became adipocyte-like when cultured under the appropriate conditions [2], with an estimated 95-100% of the adherent cells being positive for lipid as determined by Oil Red O staining (Figure 1D). Finally, neuronal differentiation could be induced using an established differentiation cocktail [2] and almost all adherent cells were positive for neuron specific midweight neurofilament (Figure 1E), unique cell surface marker of nerve cells.

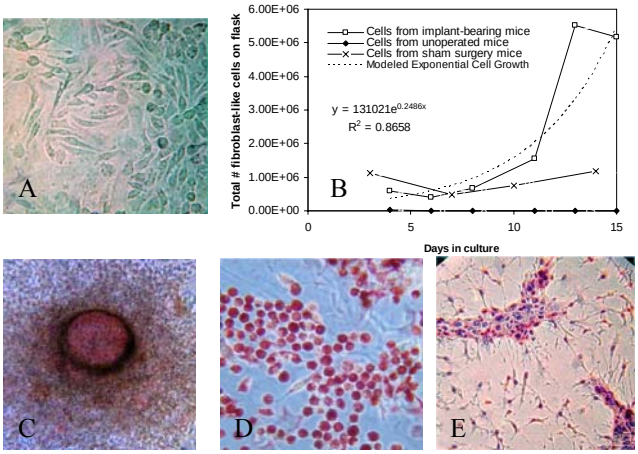

Fig 1. Undifferented cells in culture (A); Growth curves of the cells (B); Alizarin Red S staining showing calcium contents (C); Oil Red O staining showing adipocytes (D); Neuron-like cells stained with H&E (E).

The recruitment kinetics of mesenchymal stem cells (SSEA4+/CD45-) was then studied. We found that mesenchymal stem cells appeared in the peritonea as early as 12 hours following implantation (Figure 2). The numbers of mesenchymal stem cells continued to increase and achieved a plateau around 4 days before disappearing from the peritonea.

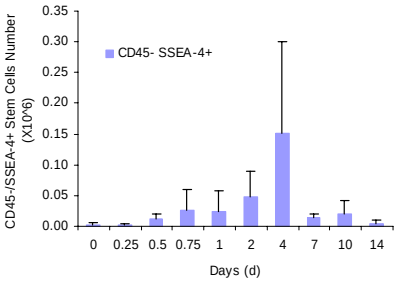

Fig 2. Inflammatory cells (CD45+) and MSCs (CD45-/SSEA-4+) numbers in mice peritonea lavage fluid.

Conclusions: Our results suggest that biomaterial implantation can trigger the recruitment of multi-potent stem cells. These cells express many mesenchymal stem cells markers and can be differentiated into osteogenic, neuronal and adipocyte-like cells with proper stimulation. We believe that biomaterial implantation may serve as a convenient way to recruit a great number of autologous mesenchymal stem cells for tissue engineering and stem cell therapies.

References:

1. Tang L. J Exp. Med. 1993; 122:292-300.
2. Gonzalez R. Biochem Biophys Res Commun. 2007; 362:491-497.