**Heparinized Collagen Loaded with Stromal-Derived Factor 1α Increases Hematopoietic Stem Cell Adherence**

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**Statement of Purpose:** In adults the hematopoietic system is localized in the bone marrow. New blood cells are produced there from hematopoietic progenitor cells (HPCs). In niches the HPCs interact with stromal cells, extracellular matrix components and cytokines. The chemokine Stromal-Derived Factor 1α (SDF-1α) is thought to play an important role in the retention of HPCs in their niches. In this study we describe the effect of SDF-1α on HPC binding to heparinized collagen films.

**Methods:** Collagen films with a thickness of approximately 50 µm were prepared from insoluble type I collagen (bovine achilles tendon, Sigma, St. Louis, USA) and were crosslinked and heparinized using EDC/NHS in a MES buffer. The films were heparinized with an EDC/NHS-activated heparin sodium salt (porcine mucosa, Sigma, St. Louis, USA). Circular samples were punched, rinsed with 70% ethanol, fixed in the wells of a culture plate and incubated in a solution of penicillin and streptomycin. Before cell seeding, the films (d =1.5 $cm^2$) were incubated once more overnight with a PBS solution with or without 2.22 µg/ml SDF-1α (R&D systems, Minneapolis, USA).
$^3$H-labelled heparin sodium salt was used to determine the heparin content of the films. The radioactivity of the samples was measured using a 1410 Winspectral liquid scintillation counter (Wallac, Türku, Finland).
$^{125}$I-labelled SDF-1α was used to determine the amount of the chemokine bound and released by the films. The radioactivity was determined using a Compugamma 1282 γ-counter (LKB, Stockholm, Sweden).
Cryopreserved human $CD34^+$ cells from peripheral blood were cultured in medium consisting of IMDM (with GlutaMAX I), BSA, penicillin, streptomycin, insulin, holo-transferrin, TPO, IL-3, SCF, Flt2/Flt3-ligand. The cells were seeded at a density of $4.5 \cdot 10^4$ cells/$cm^2$ in a 24 well plate and cultured at 37˚C in a humidified 5% $CO_2$ atmosphere.

**Results / Discussion:** At the described conditions 26 ± 3 microgram heparin was immobilized per milligram of collagen. Heparinized collagen films bound 25 ng SDF-1α per milligram of film, while the collagen films and the TCPS reference surfaces bound 18 gram per milligram and 21 ng per $cm^2$, respectively.
About 20% of the SDF-1α was released from the films within the first hour, this corresponded to 39 ng/ml for the heparinized film.
The effect of surface adsorbed SDF-1α on cell binding was investigated by comparing the number of adherent cells on surfaces without SDF-1α (neither on the surface nor in the medium) and SDF-1α pre-incubated surfaces. SDF-1α was added only to the culture medium as well (50 ng/ml). After one hour the heparinized surfaces bound the highest amount of cells (figure 1). The amount of progenitor cells bound to the surface increased on SDF-1α loaded heparinized films and especially on SDF-1α loaded collagen films, as shown in figure 1.

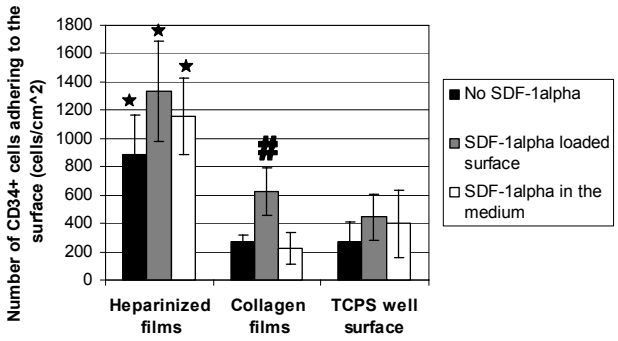


*Figure 1. Number of $CD34^+$ hematopoietic progenitor cells adhering to the surface, one hour after seeding, determined from three independent experiments performed in duplicate (± S.E.M.). * Indicates a significant difference compared to both collagen and TCPS ($p<0.1$). # Indicates a significant difference compared to the situation without SDF-1α and to the situation with SDF-1 α added to the medium ($p<0.05$).*

CFU-assays performed on the adherent cells showed that a part of the erythroid (CFU-E) progenitor population bound to the SDF-1α loaded heparinized collagen film as illustrated in figure 2.

*Figure2. CFU-E colonies on a heparinized collagen film preincubated with SDF-1α (right picture) and on TCPS (left picture). CFU assays performed on adherent cells after 3 weeks of culture. Original magnification 100x*

During 2 weeks of culture with the same cells on SDF-1α loaded heparinized collagen films an 8 to 9 fold cell expansion was reached.

**Conclusions:** The results show increased hematopoietic progenitor binding onto SDF-1α pre-incubated heparinized collagen films. Moreover, our data indicate an increased ability of SDF-1α to stimulate cell binding when adsorbed onto a surface as compared to SDF-1α in solution. The cells adhering to the heparinized collagen surfaces were shown to be mainly erythroid progenitors.