Surface-Aminated Electrospun Poly(ethersulfone) Fibers Enhances *Ex Vivo* Expansion of Human Umbilical Cord Blood Hematopoietic Progenitor ($CD34^+$) Cells

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Introduction:

Ex vivo expansion of hematopoietic stem/progenitor cells (HSPCs) is critical for bone marrow transplantation in treating a variety of hematologic disorders and as a supportive therapy for malignant diseases. HSPCs are generally regarded as a non-adherent cell. Therefore, research on expansion has been primarily focused on medium compositions, growth factor and cytokine formulations [1]. Several studies suggest that substrate might play a role in affecting HSPC proliferation outcome [2–3]. We hypothesize that aminated substrates will act synergistically with cytokine supplementation to improve *ex vivo* expansion of HSPCs. In this study, we investigate the effect of different surface amino groups on HPC proliferation, in comparison with unmodified and carboxylic substrate.

Methods:

Polyethersulfone nanofiber mesh was prepared by electrospinning from a 20 wt% solution in dimethylsulfoxide. The average diameter of PES nanofiber was 529 ± 144 nm. The nanofiber meshes was functionalized by UV-initiated poly(acrylic acid) (PAAc) grafting [4]. Meshes with surface COOH density between 100 – 300 nmol/cm^2 were used. The PAAc-grafted PES nanofiber mesh was further conjugated with excess hexamethylenediamine (HeDA) or ethylenediamine (EtDA) through a 2-step carbodiimide cross-linking method. Subsequently, human cord blood $CD34^+$ hematopoietic progenitor cells (HSPCs, AllCell Inc., USA) were seeded onto EtDA or HeDA modified PES nanofiber scaffolds at 600 HSPCs per scaffold, and maintained in StemSpan™ serum-free medium (StemCell Tech.) supplemented with a cytokine combination of SCF, Flt3, TPO, IL3 and LDL, for a period of 10 days at 5% CO_2, 37°C. Control substrates included tissue culture polystyrene surface (TCPS), unmodified PES and PAAc-grafted PES nanofiber meshes. After 10 days, all expanded cells were harvested and analyzed through hematocytometer cell counting (Fig. 1), CD-marker flow cytometry analysis (Fig. 1), as well as for colony-forming cell (CFC) assay (Table 1).

Results and Discussion:

Fig. 1 shows that both the total cell and $CD34^+CD45^+$ cell fold expansion were distinctly different on the different scaffold conditions. Cells cultured on unmodified PES and PES-AAc scaffolds do not proliferate well. Cells cultured on TCPS proliferated by 674-fold, but yielded a low 6.2% $CD34^+$ fraction (42-fold $CD34^+$ cell expansion). In contrast, cells cultured on PES-EtDA scaffold expanded 659-fold and retained 25% $CD34^+$ fraction. This corresponded to 163-fold $CD34^+$ cell expansion. Interestingly, cells cultured on PES-HeDA scaffold yielded a lower total cell fold expansion (209-fold), but with a high 39% $CD34^+$ fraction (81-fold $CD34^+$ cell expansion). CFC assay (Table 1) confirmed that the expanded cells contained high frequencies of CFUs, particularly CFU-GEMMs. The highest CFU expansion was found on the aminated scaffolds.

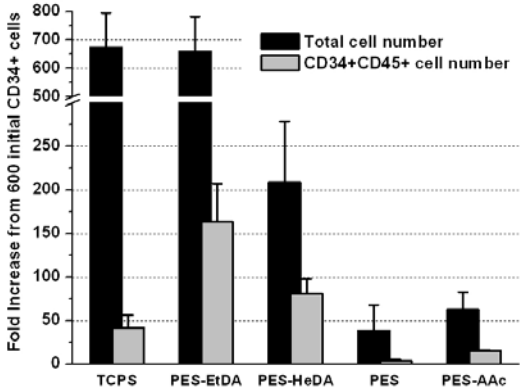

Fig. 1: Fold-expansion of total nucleated cells and $CD34^+CD45^+$ cells following the 10-day expansion culture on various surfaces. Values are the mean ± SD of 2 to 5 experiments, each conducted with 4 to 12 replicates.

Table 1: CFU number per 100 initial unexpanded HPCs.

Culture surface	CFU number per 100 initial unexpanded CD34+ cells			
	CFU-GEMM	CFU-GM	BFU-E	CFU total
TCPS	594 ± 156	1491 ± 184	525 ± 151	2610 ± 446
PES-EtDA	1362 ± 229	1298 ± 139	469 ± 202	3129 ± 261
PES-HeDA	1489 ± 176	1232 ± 180	744 ± 171	3465 ± 264
PES	0	138 ± 26	0	138 ± 26
PES-AAc	0	124 ± 45	0	124 ± 45

CFUs were generated after 14 days of culture, using the cells from the 10-day HSPC expansion cultures on various surfaces. Values are the mean ± SD of 2 experiments (n=3).

Conclusions:

The results show that aminated PES nanofiber scaffolds support the expansion of $CD34^+$ cells and CFUs, as compared to TCPS, unmodified, and carboxylic substrate. The expansion mechanism for aminated surfaces remains to be investigated. Future experiments will include lineage marker analysis to further characterize the effects of aminated scaffolds on HSPC expansion.

References:

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