COLLAGEN SCAFFOLDS FOR HEMATOPOIETIC PROGENITOR CELL EXPANSION AND CONTROLLED DIFFERENTIATION

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Summary

Controlled expansion and differentiation of primitive hematopoietic progenitor cells (HPCs) could allow the transfusion of specific blood cells in large quantities in patients treated with high dose chemo- or radiotherapy. In this study, we introduce the development of a novel, tissue engineering inspired approach to HPC expansion. We present the development of porous crosslinked collagen structures that allow proliferation of HPC-like cells and that can be further modified to create an optimal environment for HPCs to expand and differentiate.

Introduction

In adults, the hematopoietic system is localized in the bone marrow. This system contains a small population of self-renewing cells that are able to differentiate into distinct blood cells. These HPCs are destroyed in people receiving a high dose chemo- or radiotherapy. In these cases, bone marrow (BM) or peripheral blood stem cell (PBSC) transplantation is needed. Expansion and controlled differentiation of the HPCs could reduce the number of BM or PBSC harvest procedures while allowing the transfusion of the required cells in large quantities.

The bone marrow contains niches in which HPCs are localized in a specific microenvironment. In these niches, the cells interact with stromal cells, extracellular matrix components and cytokines [1]. In this project, the aim is to mimic these niches in porous collagen structures that provide a three-dimensional surrounding for the HPCs. In later stages of the project, these scaffolds will be surface modified with bioactive molecules found in the bone marrow [2,3]. In this paper, we describe the processing of collagen type I into porous sponges and beads that allow proliferation of HPC-like cells and can be modified with the bioactive molecule heparin.

Experimental methods

Collagen scaffolds were prepared with three methods. Porous sponges were prepared from insoluble type I collagen from bovine achilles tendon. Collagen (1% w/v) was dispersed after it was swollen overnight in 0.05 M acetic acid solution at 4 °C. The resulting slurry was filtered and air was removed under vacuum. The suspension was cast in polystyrene flasks and frozen at different temperatures (−20, −35 and −196 °C). The frozen suspensions were subsequently lyophilised, resulting in porous collagen structures. The sponges were crosslinked using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS). In order to obtain an open pore structure, these procedures were carried out in a 95% ethanol solution. After crosslinking the sponges were washed and lyophilised.

Porous beads were prepared by electrospraying of a collagen suspension into liquid nitrogen. The collagen suspension was prepared as described above. The frozen beads were collected from the liquid nitrogen and lyophilised, resulting in porous beads. The porous beads were crosslinked using EDC and NHS as described above.

Solid collagen beads were prepared by emulsification. Acid-soluble collagen type I from calf skin was dissolved overnight in 0.5 M acetic acid solution at 4 °C. The cooled collagen solution was neutralized and added drop-wise to stirred paraffin oil at 37 °C. The emulsion was left stirring for 3 h in order to allow the collagen to reconstitute as beads. Then, water was added and the beads were removed from the aqueous phase. The beads were washed with a 70% ethanol solution and water before the beads were crosslinked using EDC and NHS as described above. Sieves were used to obtain a narrow range of bead sizes.

For preliminary modification experiments, the collagen structures were modified using heparin sodium salt (Bufa Chemie, Castricum, the Netherlands). The crosslinked collagen structures were first equilibrated with 0.05 M MES buffer for 30 min. The carboxylic acid groups were activated by adding EDC and NHS to a 2% (w/v) solution of heparin in 0.05 M MES-buffer (pH 5.5–6.0) at a molar ratio of EDC:NHS:Hep-COOH of 0.4:0.24:1.0. After preactivation for 10 min, 1 g of crosslinked collagen was added to 188.3 ml of EDC/NHS-activated heparin solution. After 2 h of reaction, the heparinized collagen was washed with 0.1 M Na_2HPO_4 for 2 h, four times for 24 h with 4M NaCl and three times for 24 h with distilled water.

The shrinkage temperature (T_s) of (crosslinked) collagen was determined using Differential Scanning Calorimetry (DSC). The onset of the endothermic peak was recorded as the temperature at which the collagen sample undergoes thermal denaturation. This value is used as a measure of the crosslink density. A Hitachi S800 scanning electron microscope (SEM) was used to examine the morphology and pore size of the scaffolds.

Cell culture experiments with the KG1a leukaemia cell line were used as a model to evaluate HPC growth on the prepared materials. These cells were seeded at a density of 15 000 and 3750 cells per well in a 96-well roundbottom plate with 200 μl IMDM containing 20% FBS and were cultured for 1 week.

Alcian blue staining was used for localisation of the heparin in crosslinked collagen samples. Sections of the samples were incubated in a 3% (v/v) acetic acid solution for 3 min under vacuum and then stained by incubation in a 2% (w/v) solution of Alcian blue in 3% (v/v) acetic acid for 30 min under vacuum. After washing four times with demineralised water for 15 min, the samples were evaluated visually.

Results and discussion

We are able to produce highly porous sponges and porous or solid beads using collagen type I. The characteristics of the structures used for the KG1a cell culture are shown in Table 1.

Table 1
Collagen scaffold characterization

Scaffold type	Size (μm)	T_{freeze} (°C)	Pores (μm)	T_s (°C)
Solid beads	500	–	–	72±1
Porous sponges	–	−196	15±5	68±2
Porous sponges	–	−35	43±6	67±1
Porous sponges	–	−18	149±4	67±1
Porous beads	870	−196	6±2	74±1

These techniques allowed us to tune the properties of the sponges and beads. Examples of the obtained porous sponges can be seen in Figs. 1 and 2.

Light microscopic analysis of the cell culture experiments with the KG1a cell line showed that these HPC-like cells proliferate when they are cultured on the collagenous sponges and beads. A representative image of the cells on a bead is shown in Fig. 3.

The modification of the beads and sponges with heparin resulted in a lower amount of free amino groups, indicating that heparin was covalently bound (not shown here). Staining with Alcian blue confirmed that we are able to homogeneously modify the scaffolds with heparin when the porous structures are interconnected. This can be seen in Fig. 4, where sample A is a scaffold with poor interconnectivity and C a highly interconnected scaffold.

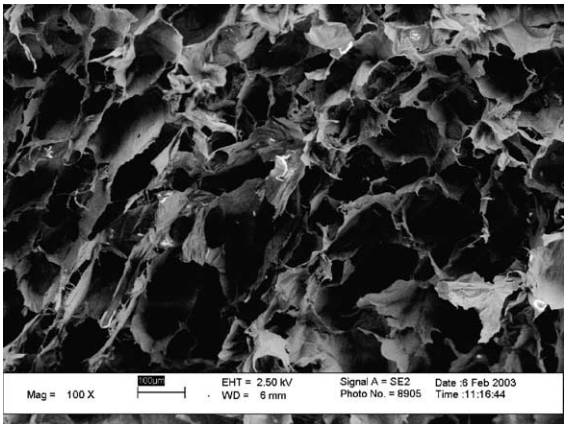

Fig. 1. Collagen sponge with large average pore size at 100× magnification.

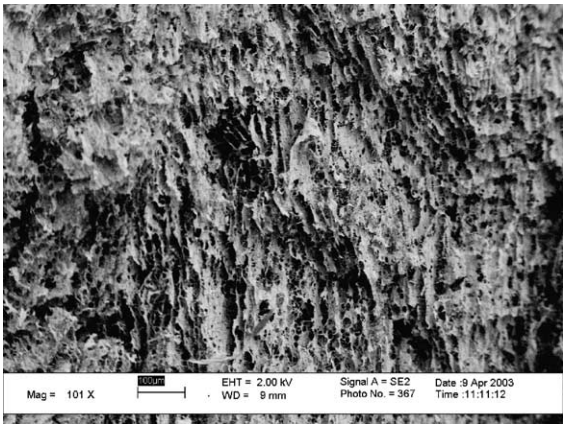

Fig. 2. Collagen sponge with small average pore size at 100× magnification.

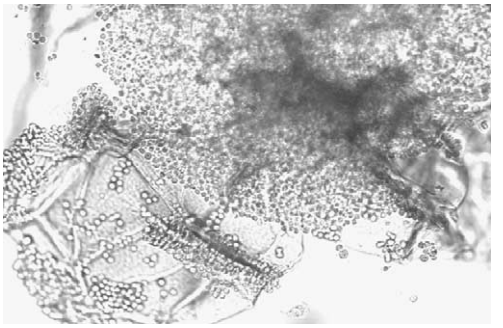

Fig. 3. KG1a cells on collagen beads, day 3 (100× magnification).

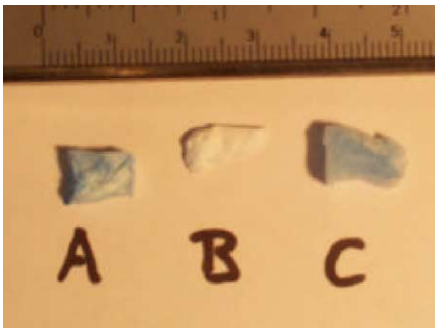

Fig. 4. Alcian blue stained heparinized scaffold sections: A—small average pore size (Fig. 2), B—not heparinized control scaffold and C—large average pore size (Fig. 1).

Conclusion

We are able to produce collagenous scaffold structures that support the proliferation of the hematopoietic cell line KG1a and that can be modified with bioactive molecules like heparin. These structures will now be used for cell culture with HPCs ($CD34^{+}$) obtained from peripheral blood. The interaction of the heparinized structures with chemo- and cytokines will then be investigated with radiolabeled molecules.

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