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ECM-based macroporous sponges release essential factors to support the growth of hematopoietic cells

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ABSTRACT

The success of hematopoietic stem cells (HSCs) transplantation is limited due to the low number of HSCs received from donors. *In vivo*, HSCs reside within a specialized niche inside the 3D porous spongy bone. The natural environment in the niche is composed of structural proteins, glycosaminoglycans (GAGs) and soluble factors that control cells fate. However, the designed scaffolds for *in vitro* culture do not fairly recapitulate this microenvironment and cannot efficiently control HSCs fate. Here we report on the development of new omental ECM-based 3D macroporous sponges for hematopoietic cell culture. The scaffolds' structure, porosity and stability were characterized and optimized. Analysis of the biochemical content revealed that they were composed of collagens and GAGs, including sulfated GAGs. This morphology and composition enabled growth factors interaction with the sulfated GAGs, as indicated by the high loading capacity and release profile of three different hematopoietic niche factors. Finally, the ability of the ECM-based scaffolds to efficiently support the growth of hematopoietic cells by releasing stem cell factor (SCF) was demonstrated.

1. Introduction

Transplantation of hematopoietic stem cells (HSCs) has the potential of treating hematologic disorders such as different types of leukemia, immune deficiencies and autoimmune diseases. Unfortunately, the success of HSCs transplantation is limited due to the low number of HSCs received from the donor. This results in a significant delay in hematopoiesis, accounting for the relatively high transplant-related mortality rate [1]. Successful HSC expansion studies have spanned over the last three decades. However a routine method for efficient *ex-vivo* expansion of HSCs is still an unmet need [2].

In vivo, HSCs reside within a specialized niche inside the spongy bone. In this 3D porous structure, glycosaminoglycans (GAGs) and proteins, including collagen type I, fibronectin and laminin, provide physical support and topographical cues to the HSCs [3]. Soluble factors, such as stem cell factor (SCF), Fms-related tyrosine kinase 3 ligand (Flt3L) and vascular endothelial growth factor (VEGF) electrostatically interact with sulfated GAGs, which act as a protein depot. According to the physiological need these growth factors are released to initiate signaling pathways determining cell fate [3–6].

One of the goals in tissue engineering is to design 3D biomaterials that closely mimic the natural ECM, in order to properly maintain cell growth [7–9]. However, the detailed biochemical composition of the natural matrix, which best foster cellular organization is still not completely understood. Therefore, synthetic recapitulation of this microenvironment is a complicated task [8,10,11]. Consequently, decellularized matrices were developed and used in order to supply the cells with the essential biochemical cues that efficiently support their growth. During decellularization process, cells are gently removed from a harvested tissue or organ by chemical, physical and biological methods [8], while the essential biomolecules are preserved [12]. Recent works have demonstrated the engineering of functional cardiac, hepatic and lung tissues by using different decellularized matrices from the heart, liver and the omentum [13–17].

The omentum is a highly vascularized adipose tissue that extends from the stomach overlying the abdomen [18]. Its ECM is rich with different types of collagens, hyaluronan, sulfated GAGs and growth factors [19,20], making it an ideal microenvironment for stem cells, with proven regenerative capabilities [21,22]. In a recent work our group has demonstrated the potential of omentum-based scaffolds or hydrogels to engineer cardiac tissues [22–24]. We have further optimized the decellularization process of the tissue in order to obtain a better balance between complete decellularization and preservation of essential ECM components such as GAGs [23,24]. Here we report on the development

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of new omental ECM-based 3D macroporous sponges. The new cell-supporting scaffolds are based on the ability to liquefy the decellularized omentum [25], casting and lyophilizing to create homogenous and porous microenvironment. We have investigated the physical and biochemical properties of the obtained scaffolds and assessed the potential of the preserved sulfated GAGs (s-GAGs) to serve as a depot for essential growth factors related to the bone marrow niche. We have further demonstrated the potential of the scaffolds to slowly release the factors into the cellular microenvironment, serving as an effective platform for hematopoietic cell growth.

2. Materials and methods

All materials were purchased from Sigma (Rehovot, Israel) unless stated otherwise.

2.1. Decellularization

Omenta of healthy pigs were purchased from the institute of animal research in Kibutz Lahav, Israel. All steps of incubations and washes were obtained at room temperature on an orbital shaker unless noted otherwise.

Fresh omentum was washed with phosphate buffered saline (PBS) in order to deplete blood and debris. The tissue was then agitated in a hypotonic buffer of 10 mM Tris 5 mM Ethylenediaminetetraacetic acid (EDTA) and 1 μM phenylmethanesulfonyl-fluoride (PMSF) at pH 8.0. Next, the tissue went through three cycles of freezing (−80 °C) and thawing (37 °C) using the same buffer. After the last thawing the tissue was dehydrated by 70% ethanol wash, followed by three 100% ethanol washes. Lipid extraction was then conducted by washing the omentum with 100% acetone following 24 h agitation in 60/40 (v/v) hexane:acetone solution. The defatted tissue was rehydrated, washed and incubated in 0.25% Trypsin–EDTA (Biological Industries, Kibbutz Beit-Haemek, Israel) solution overnight for cell removal. The tissue was then washed thoroughly with PBS. For nucleic acids elimination the tissue was then incubated in 1.5 M sodium chloride solution for 24 h. Next, the processed omentum was washed with 50 mM Tris 1% triton-X100 solution at pH 8.0 for 1 h. Finally, the tissue was washed with PBS and with double distilled water before freezing and lyophilizing.

2.2. Gel formation

The lyophilized decellularized omentum was ground into powder using Wiley Mini-Mill. Different quantities of the powder were farther digested by 1 mg/ml pepsin solution in 0.1 M HCl, creating different concentrations (0.5%, 1% and 1.5% w/v). the digestion process was conducted in room temperature, using slow stirring. The digestion was terminated by pH adjustment to 7.2–7.4 using NaOH.

Measured volumes of solution were casted in round wells and incubated in 37 °C for gelation.

2.3. Porous scaffolds formation

Three different freezing regimes were used in order to obtain different porosity. The gels were either frozen in liquid nitrogen, placed inside a −20 °C freezer, or slowly frozen in an isopropanol bath inserted in −20 °C freezer overnight. All frozen gels were lyophilized under the same conditions. The lyophilized scaffolds were incubated overnight in PBS at 37 °C and then washed with double distilled water. The washed scaffolds were lyophilized again.

2.4. DNA staining and quantification

For nucleic acid detection, small pieces from the native and the processed tissues were stained with 5 μg/ml Hoechst 33258 for 3 min, followed by PBS washes. The samples were visualized using an inverted fluorescence microscope (Nikon Eclipse TI).

DNA was extracted from three scaffolds and three random 25–30 mg dried samples of the native tissue using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manual guide. The obtained DNA was quantified by measurements of the O.D at 260 nm wavelength using spectrophotometer (Nanodrop 1000, Thermo Scientific).

2.5. Scanning electron microscopy

For scanning electron microscope (SEM) imaging, cross sections of the lyophilized scaffolds were sputter-coated with gold and then observed under SEM (Jeol JSM840A). The properties of the pores from three different scaffolds measured with ImageJ program (NIH).

2.6. Sulfated glycosaminoglycan quantification

The sulfated glycosaminoglycans (GAGs) in the native omentum and the processed scaffolds were quantified using the Blyscan™ sulfated GAG assay kit (Biocolor Ltd., Carrickfergus, UK) according to the manufacturer instructions. Briefly, the tissues were digested with papain. The digested solutions were centrifuged and the supernatants were examined with dimethylmethylene blue. Overall, three samples were picked for each assay.

2.7. Histology

Wet scaffolds were placed inside optimum cutting temperature (O.C.T.) embedding medium (Tissue-Tek, Sakura, Japan) and snap frozen in liquid nitrogen. Sections of 20 μm were obtained using a Cryotome™ FSE (Thermo scientific) and affixed to X-tra® adhesive glass slides (Leica Biosystems, Wetzler, Germany). The slides were stained with Alcian-blue and Fast-red (Merck) for GAG imaging. Masson's trichrome (Bio-Optica, Milano, Italy) for cell and collagen detection staining were performed according to the manufacturer's guidelines.

2.8. Measurements of growth factors loading and release from the scaffolds

For the sustained release assay, triplicates of lyophilized scaffolds or empty wells were loaded in non-binding 96-well plates (Corning) with 50 ng of stem cell factor (SCF), FMS-like tyrosine kinase (Flt3-L) or vascular endothelial growth factor (VEGF) all purchased from PeproTech Asia. The loaded scaffolds were incubated for 2 h at 37 °C, and then washed once with PBS-0.1% bovine serum albumin (BSA). The scaffolds were incubated in PBS-0.1% BSA at 37 °C for 10 days. The medium was collected and replaced at different time points (1, 2, 4, 7 and 10 days) and preserved at −20 °C for analysis.

The samples were thawed, diluted and examined by enzyme linked immune-sorbent assay (ELISA) for the different growth factors (ELISA kits for Flt3-L and SCF were purchased from PeproTech Asia, duo-set ELISA kit for VEGF was purchased from R&D Systems®).

For the loading assay, triplicates of lyophilized scaffolds were loaded with 50, 100, 200 or 400 ng of SCF, Flt3-L or VEGF. The loaded scaffolds were incubated for 2 h at 37 °C and than washed once with PBS - 0.1% BSA. The scaffolds were digested using 2.5 mg/ml collagenase type I in PBS - 0.1% BSA solution at 37 °C for 1 h. the digested samples were examined by ELISA for the different growth factors.

2.9. Erythroid myeloid lymphocyte (EML) cells growth within the scaffolds

EML cells were purchased from ATCC® and handled as instructed. Briefly, the cells were cultured in humidified incubator with 5% CO_2 at 37 °C, in Iscove's modified Dulbecco's medium (IMDM, from ATCC®), containing 200 ng/ml mouse SCF (PeproTech Asia) and 20% heat-inactivated fetal bovine serum (Biological industries).