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Human-derived extracellular matrix from Wharton's jelly: An untapped substrate to build up a standardized and homogeneous coating for vascular engineering

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ABSTRACT

One of the outstanding goals in tissue engineering is to develop a natural coating surface which is easy to manipulate, effective for cell adhesion and fully biocompatible. The ideal surface would be derived from human tissue, perfectly controllable, and pathogen-free, thereby satisfying all of the standards of the health authorities. This paper reports an innovative approach to coating surfaces using a natural extracellular matrix (ECM) extracted from the Wharton's jelly (WJ) of the umbilical cord (referred to as WJ-ECM). We have shown by atomic force microscopy (AFM), that the deposition of WJ-ECM on surfaces is homogenous with a controllable thickness, and that this easily-prepared coating is appropriate for both the adhesion and proliferation of human mesenchymal stem cells and mature endothelial cells. Furthermore, under physiological shear stress conditions, a larger number of cells remained adhered to WJ-ECM than to a conventional coating such as collagen – a result supported by the higher expression of both integrins α2 and β1 in cells cultured on WJ-ECM. Our data clearly show that Wharton's jelly is a highly promising coating for the design of human biocompatible surfaces in tissue engineering as well as in regenerative medicine.

Statement of Significance

Discovery and design of biomaterial surface are a hot spot in the tissue engineering field. Natural matrix is preferred to mimic native cell microenvironment but its use is limited due to poor resource availability. Moreover, current studies often use single or several components of natural polymers, which is not the case in human body. This paper reports a natural extracellular matrix with full components derived from healthy human tissue: Wharton's jelly of umbilical cord. Reconstituting this matrix as a culture surface, our easily-prepared coating provides superior biocompatibility for stem and mature cells. Furthermore, we observed improved cell performance on this coating under both static and dynamic condition. This novel human derived ECM would be a promising choice for regenerative medicine.

1. Introduction

The main goal of the tissue engineering triad is to create combinations of cells, scaffolds and environment (i.e. soluble mediators and/or mechanical stresses). For vascular tissue engineering, finding appropriate cells and scaffolds remain the two major challenges. Stem cells, and in particular, mesenchymal stem cells (MSCs), have emerged as the most promising source of suitable cells as a result of their good availability and ability to differentiate into vascular cells, as well as their immunosuppressive properties which are particularly interesting for allogeneic applications [1,2]. As to the scaffold, synthetic materials are most commonly used for engineered vascular grafts, but they are poor substrates for cells. Therefore, substantial effort has been made to develop new coatings, ranging from synthetic polymers to isolated natural extracellular matrix (ECM) to: (i) provide a solid base for cell anchorage to allow resistance to blood flow; (ii) favor endothelial differentiation of stem cells; and (iii) facilitate cell expansion to mimic the endothelial layer [3,4].

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Synthetic polymers have the advantage of being precisely tailored, and they can be combined with other biological interfaces, allowing for the construction of desired tissues [5]. However, those which have been studied to date have often exhibited poor biocompatibility and/or immunogenicity, limiting their clinical use. In the case of ECM, decades of extensive research on its structure and biological influence on cell behavior and fate have shown that its physical properties (elasticity, stiffness, resilience to the cellular environment, etc.), specific chemical signals exhibited by its peptide epitopes, and the nanoscale topography of microenvironmental adhesive sites, are all relevant to the design of biomimetic surfaces [6]. Nonetheless, current studies usually exploit one or several purified ECM components to construct surfaces [7], a practice which does not faithfully replicate the native ECM entire environment [8].

Complete ECM derived from human tissue could be an ideal source for natural matrices. In this context, tissues such as placenta and umbilical cord which are obtained from non-pathological medical procedures, could provide a good supply. For example, placenta has recently been treated using a chemical procedure (urea solubilization) to furnish a complex of ECM for tissue engineering [9]. Wharton's jelly of human umbilical cord contains various types of collagen and glycosaminoglycan, as well as growth factors and/or cytokines synthesized by mesenchymal stem cells, and thus may be viewed as a natural source for ECM-based coating [10]. Indeed, Hao et al. previously showed that mechanically-obtained ECM from Wharton's jelly can support MSC culture [11].

Here we describe a novel enzymatic method to isolate complete ECM from Wharton's jelly (this material will be referred to hereafter as WJ-ECM), which boasts many benefits relative to existing procedures. The most important advantages of our coating are its human tissue origins and a method of preparation that does not involve chemicals which are toxic to cells. To test our material, we prepared, characterized, and determined the *in vitro* response to WJ-ECM of MSCs and endothelial cells, which are currently the two most-used cell sources in vascular tissue engineering, under both static and dynamic conditions. Our data show that WJ-ECM-derived coating enhanced proliferation of both human MSCs (hMSCs) and human umbilical vein endothelial cells (HUVECs). This new surface also exhibits improved cell resistance to flow under shear stress, a phenomenon which is of particular interest in vascular tissue engineering. The role of integrins in the possible mechanism underlying cell retention under shear stress was also investigated.

2. Methods

2.1. Preparation of WJ-ECM coating

Fresh human umbilical cords were obtained after full-term births with informed consent using the guidelines approved by the Cell Therapy Unit of the University Hospital Center (CHRU) of Nancy (Authorization number: TCG/11/R/011). Briefly, whole umbilical cord was washed 3 times with Hank's balanced salt solution (HBSS) and cut into 5-cm explants, and Wharton's jelly was carefully isolated without taking umbilical cord membrane or vessels. The WJ of umbilical cord from 3 separate donors was then pooled prior to sample homogenization. The isolated WJ was further cut into 0.5-cm pieces using sterile surgical scissors. The WJ was then digested in trypsin (0.025%, Bioblock, 1158-0626, France) at 37 °C for 24 h with magnetic stirring (ratio of wet WJ/trypsin was 1 g: 5 ml), the stirred suspensions were centrifuged at 16,000*g* for 10 min, and the collected supernatants were filtered (100 μm filter). Fetal bovine serum (FBS) (Dutscher, S1900) was added into the solution (final concentration of 10%) to inhibit the trypsin reaction. The concentration of WJ (mg/ml) was calculated using lyophilized WJ tissues dissolved in final phosphate buffered saline (PBS). The obtained WJ-ECM solution was conserved at 4 °C for further use. To coat culture surfaces, the freshly-obtained or conserved WJ-ECM was deposited onto glass coverslips which had been carefully pre-treated with SDS-HCl [12]. The glass coverslips coated with WJ-ECM were incubated for 12 h at 37 °C, then washed twice with culture medium.

2.2. Characterization of the surface coating by AFM

Before each measurement, fresh samples were extensively rinsed with milli-Q water and then slowly and completely dried with nitrogen. AFM experiments were carried out using a MFP3D-BIO instrument (Asylum Research Technology, Atomic Force F&E GmbH, Mannheim, Germany). Silicon nitride cantilevers of conical shape were purchased from Olympus (OTR-400 PSA, Bruker-nano AXS, Palaiseau, France). The spring constants of the cantilevers measured using the thermal noise method were found to be 0.020–0.024 nN/nm. Experiments were performed both in air and in PBS buffer at room temperature. AFM images were recorded at room temperature (20 °C) in contact mode. The applied force between the tip and the surface was carefully monitored and minimized at approximately 0.2 nN for experiments performed in aqueous medium. All images were collected with a resolution of 512 × 512 pixels at a scan rate of 1 Hz. The nanoindentation method was used to quantify the mechanical properties. Young's modulus of the thin films was calculated from force vs. indentation curves. Mechanical properties were obtained by recording a grid map of 32-by-32 force curves at different locations on the thin deposits over the 10 μm × 10 μm surface area. The maximal loading force was 0.5 nN, the piezodrive was fixed to 1 μm and the approach rate was 1000 nm s^{-1}. The Young's modulus was estimated from the analysis of the approach curves according to the Sneddon model [13].

$$F = \frac{2E \cdot \mathrm{Tan}(\alpha)}{\pi(1-\nu^2)} R^{1/2} \delta^2 \cdot f_{\mathrm{BECC}}$$

where δ is the indentation depth, ν the Poisson coefficient, R the curvature radius of the AFM-tip apex and f_{BECC} is the Bottom Effect Cone Correction function that takes into account the presence of substrate stiffness [14]. All the force curves were analyzed by means of an automatic Matlab algorithm described elsewhere [15].

2.3. Biomolecular composition analysis

WJ-ECM solution (10 mg/ml) was analyzed using a human angiogenesis array kit (R&D systems, ARY007, France) according to the manufacturer's protocol, and the pixel density of detected spots was measured with Image J software.

2.4. Culture of hMSC and HUVEC cells

To isolate hMSCs from umbilical cord (UC), Wharton's jelly was sliced into 5-mm explants after removing the umbilical vessels and the umbilical outer layer membrane, and the slices were subsequently attached and cultured in minimum essential medium Eagle-alpha modification (α-MEM) (Lonza, BE12-169F) supplemented with 10% FBS, Fungizone® (100 mg/ml, Fisher, 11520496), penicillin (100 IU/ml, Sigma, P0781) and L-glutamine (200 mM, Sigma, G7513) on culture plates. The medium was changed every 3 days, and hMSC populations appeared as outgrowths from the UC fragments at day 6. After 15 days, the UC fragments were discarded, and the cells were passaged with trypsin and expanded until they reached sub-confluence. To isolate HUVECs,