Material Sciences , 2018, 8(5), 595-602
Published Online May 2018 in Hans. http://www.hanspub.org/journal/ms
https://doi.org/10.12677/ms.2018.85070

Quantitative Detection of Growth Factors in Porcine Small Intestinal Submucosa Matrix Materials

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Received: May 4 th, 2018; accepted: May 20th, 2018; published: May 28 th, 2018

Abstract

In this study, three growth factors including basic fibroblast growth factor (FGF2), transforming growth factor β1 (TGFβ1) and vascular endothelial growth factor (VEGF) in two SIS products (VIDASISTM and Biodesign®) were quantified by enzyme linked immune sorbent assay (ELISA) kit through the preliminary treatment of liquid nitrogen freezing and grinding technology which was aimed to release the growth factors from the tissue. Results showed that contents of TGFβ1, VEGF and FGF2 in VIDASISTM were 8375 ± 2125 pg/g, 5486 ± 1043 pg/g and 3990 ± 1372 pg/g respectively; while the corresponding contents in Biodesign® were 11517 ± 1331 pg/g, 5432 ± 272 pg/g and 5417 ± 947 pg/g.

Keywords

Porcine Small Intestinal Submucosa, Acellular Matrix, Transforming Growth Factor, Basic Fibroblast Growth Factor, Vascular Endothelial Growth Factor

1 Introduction

Porcine small intestinal submucosa acellular matrix (SIS, Small Intestinal Submucosa) is a membrane-like material prepared by decellularization, molding and sterilization of the submucosa of the pig small intestine. It has high biocompatibility, biodegradability and absorbability. It is widely used in the repair or reconstruction of tissues such as tendons, dura mater, abdominal wall and skin [1] [2]. The shortcomings of traditional repair surgery, such as long operation time and high tension, affect the recovery of patients. SIS materials can improve these defects to a

certain extent. The characteristics of inducing cell adhesion growth and low infection rate can prove that SIS is a good animal-derived plant into the material. The components of SIS patch mainly include type I and III collagen, fibronectin and a variety of growth factors (TGF-β, VEGF and FGF, etc.) [2] [3]. Growth factors are cytokines secreted by a variety of cells, acting on specific target cells, regulating cell division, matrix synthesis and tissue differentiation, and contributing to the enhancement of cell function and the formation of vascularization [4] [5].In body tissues, cells, growth factors, and ECM are all in a dynamic environment, and the multiple interactions among the three constitute the microenvironment for cell survival in the body [6]. They are highly active, multifunctional polypeptides or proteins, which mainly play a local role at close range. They have a very high affinity with receptors and are also extremely susceptible to inactivation due to external influences. Wound healing is a complex biological process. Growth factors are increasingly playing an important role in improving various physiological and pathological conditions, promoting tissue regeneration, repair and wound healing processes. The key lies in promoting the formation of granulation tissue. Accelerate the re-epithelialization process of the wound [7].

TGF-β is a rich bone matrix protein and a peptide anti-inflammatory growth factor. It is widely present in normal tissues and transformed cells. It has a high content in platelets and bone tissues. It is used in the process of bone formation and bone remodeling. It plays an extremely important role. It can control the proliferation, differentiation and activation of immune cells (T cells, B cells, etc.) for immune regulation, and promote wound healing and angiogenesis [8] [9] [10]. VEGF is a vascular-derived growth factor that is highly specific to vascular endothelial cells. It is synthesized and secreted by platelets, vascular smooth muscle cells, endothelial cells, osteoblasts, or tumor cells. It is essential for the formation of new blood vessels and can increase blood vessel communication. Permeability and promoting endothelial cell proliferation and other functions, it also has the effect of protecting nerves. Among them, the most research on VEGF is the most in-depth [8] [11]. FGF is a group of polypeptides with similar structural features, which are widely present in body tissues. At present, it is known that the FGF family includes at least 23 genes that can encode related structural proteins.

FGF has a strong mitogenic and proliferative activity, and can induce the formation of capillaries. Heparin is used in FGF2 extraction. Heparin combines with FGF2 to form a stable complex, which can maintain the biological activity of such growth factors. Heparin can also directly bind to FGF2 receptors [6] [8] [12].

ELISA kits are mostly used to detect growth factors in serum or plasma, and growth factors in tissues are not easy to extract and detect. This experiment uses three methods to pretreat the samples to release the active growth factors in SIS, and then pass ELISA The kit detects the content of growth factors, compares and screens out the appropriate pretreatment methods for different growth factors in SIS, and compares them with the content of growth factors in similar imported products.

2. Experimental part

DOI: 10.12677/ms.2018.85070

2.1. Main materials

SIS (VIDASISTM) was provided by Beijing Biosis Healing Biological Technology Co., Ltd.; SIS (Biodesign®) was purchased from Cook Company, USA; ELISA kit was purchased from Wuhan Yunclone Technology Co., Ltd.; Urea was purchased from Xilong Chemical Co., Ltd.; Heparin sodium was purchased from Sinopharm Chemical Reagent Co., Ltd.; Type I collagenase was purchased from Beijing Xinjingke Biotechnology Co., Ltd.

2.2. Main instruments

Refrigerated crusher (LT-100), Zhongke Netzsch (Beijing) Technology Co., Ltd.; LGJ-100F vacuum freeze dryer, Beijing Songyuan Huaxing Technology Development Co., Ltd.; HC-2518R high-speed refrigerated centrifuge, Anhui Zhongke Zhongjia Scientific Instruments Co., Ltd.; Thermo full-wave scanning multifunctional microplate reader VARIOSKAN FLASH, Thermo Fisher Company, USA.

2.3. Preparation of main reagents

1) Buffer 1: 2.5 mg/mL heparin sodium; 2 mol/L urea.

2) Buffer 2: 50 mmol/L Tris-HCl, pH 7.4; 0.36 mmol/L CaCl2.

2.4. Experimental method

2.4.1. Freezing and crushing steps

Cut the dry SIS patch material with sterile surgical scissors into small pieces less than 1 × 1 cm2 in size, so that the SIS material can enter the inside of the crusher; put the coarsely crushed SIS material on the feed table, and use a stainless steel feed rod to remove the SIS The material is fed into the feed port of the refrigerated crusher, and an appropriate amount of liquid nitrogen is continuously injected into the feed port. The SIS and liquid nitrogen are mixed at the feed port, and the negative pressure inside the powder cavity of the crusher is used to make the SIS powder and liquid Nitrogen is sucked into the crushing chamber, and the crushing temperature is −196℃; the outlet of the crushing chamber is equipped with a stainless steel screen with an aperture of 80-100 mesh. The positive pressure at the outlet is used to send the SIS powder that meets the size out of the crushing chamber. The outlet has good air permeability The pulverized SIS powder shall be collected in the aseptic cloth bag; after the liquid nitrogen supply is stopped, the SIS powder shall be quickly put into a sterile airtight tank or ziplock bag for later use, and can be opened for use after the room temperature is restored.

2.4.2. Extraction method of active growth factors

Three active growth factors were extracted by the following three methods:

Method 1: Weigh 5 mg of the crushed SIS material sample, add 1 mL of Buffer 1, homogenize at high speed and low temperature for 20-30 minutes until the liquid is colloidal, centrifuge at 14000 rpm for 30 minutes at high speed, extract the supernatant, and dilute to 1 mL , Store at 4°C.

Method 2: Weigh 5 mg of the crushed SIS material sample, add 1 mL of Buffer 1, homogenize at high speed and low temperature for 20-30 minutes until the liquid is colloidal, stir at 4°C for 24 hours, centrifuge at 14000 rpm for 30 minutes, and extract. The clear solution, dilute to 1 mL, and store at 4°C.

DOI: 10.12677/ms.2018.85070

Method 3: Weigh 50 mg of the crushed SIS material, add 8 mL of Buffer 2, and denature at 100°C for 10 min. After cooling, add 200 μg of collagenase I, enzymatically digest at 37°C for 1 h, and centrifuge to collect the supernatant; add 8 to the pellet mL Buffer 2 and 200 μg collagenase I were enzymatically digested at 37°C for 1 h, centrifuged to collect the supernatant; repeated to the fifth time to extract the supernatant; the supernatant was combined with lyophilization and collected and weighed. Weigh 5 mg of lyophilized material into 1 mL of Buffer 1, and store at 4°C.

Method 1 and Method 2 release as many growth factors as possible through different treatment methods, and Method 3 removes high-abundance substances such as collagen by enzymatic hydrolysis, and then extracts growth factors. After the three methods are centrifuged at high speed, the supernatant mainly contains growth factors and other trace proteins. Since the ELISA kit is a detection method based on the specific binding of antigen and antibody, other substances in the supernatant have almost no effect on the detection.

2.4.3. ELISA detection method

TGFβ1 and VEGF adopt double-antibody sandwich ELISA method, and FGF2 adopt competitive inhibition ELISA method.

Operate the samples processed by the three methods according to the instructions of the ELISA kit, and the operation steps are as follows (take TGFβ1 as an example):

1) Adding samples: set standard wells, sample wells to be tested, and blank wells respectively, add 100 μL of standards and samples of different concentrations in sequence, add standard buffer to the blank wells, and incubate at 37°C for 1 h after covering the microtiter plate , Discard the liquid and spin dry;

2) Add 100 μL of detection solution A working solution to each well, incubate at 37°C for 1 h, discard the liquid, and wash each well with 350 μL of washing solution for 1~2 min

And spin dry, the process is repeated 3 times;

3) Add 100 μL of detection solution B working solution to each well, incubate at 37°C for 1 h, discard the liquid, and wash each well with 350 μL of washing solution for 1~2 min

Spin to dry, repeat the process 5 times;

4) Add 90 μL of TMB substrate solution to each well and develop color at 37°C in the dark (10-20 min). Add 50 μL of stop solution to each well to stop the reaction, and shake gently;

5) Immediately use a microplate reader to measure the optical density value (O.D. value) of each well at a wavelength of 450 nm;

6) Establish a standard curve based on the standard product, substitute the formula to obtain the content of active growth factor in the sample, and then convert it to its content in the dry weight of the SIS patch sample.

3. Results and discussion

3.1. Test results

3.1.1. TGFβ1 test results

DOI: 10.12677/ms.2018.85070

According to the optical density value (O.D. value) of the TGFβ1 standard substance of different concentrations in the ELISA kit at the wavelength of 450 nm, a standard curve is made, as shown in Figure 1.

As shown in Figure 2, the content of TGFβ1 in VIDASISTM is 5391 ± 370 pg/g by method 1, and the content of TGFβ1 in Biodesign® is 6702 ± 230 pg/g; the content of TGFβ1 in VIDASISTM by method two is 8375 ± 2125 pg /g, the content of TGFβ1 in Biodesign® is 11517 ± 331 pg/g; Method 3 detects that the content of TGFβ1 in the two SIS patches is basically zero.

3.1.2. VEGF test results

According to the optical density value (O.D. value) of the ELISA kit with different concentrations of the VEGF standard at the wavelength of 450 nm, a standard curve is made, as shown in Figure 3.

As shown in Figure 4, the content of VEGF in VIDASISTM was 3974 ± 871 pg/g by method 1, and the content of VEGF in Biodesign® was 5213 ± 737 pg/g; the content of VEGF in VIDASISTM was 5486 ± 1043 pg by method two. /g, the content of VEGF in Biodesign® is 5432 ± 272 pg/g; Method 3 detects that the content of VEGF in the two SIS patches is basically zero.

3.1.3. FGF2 test results

According to the optical density value (O.D. value) of the FGF2 standard substance of different concentration in the ELISA kit at the wavelength of 450 nm, a standard curve is made, as shown in Figure 5.

As shown in Figure 6, the content of FGF2 in VIDASISTM is 3990 ± 1372 pg/g by method one, and the content of FGF2 in Biodesign® is 5417 ± 947 pg/g; the content of FGF2 in VIDASISTM by method two is 2668 ± 384 pg /g, the content of FGF2 in Biodesign® is 2887 ± 154 pg/g; Method 3 detects that the content of FGF2 in the two SIS patches is basically zero.

3.2. Discussion

In this study, three growth factors (TGFβ1, VEGF, FGF2) in the acellular matrix of the submucosa of the pig small intestine were detected. Because the growth factors are very sensitive to temperature, the sample preparation process is under low temperature conditions. Adding heparin sodium to Buffer 1 can combine growth factors with heparin to maintain and promote its activity. Heparin is a type of glycosaminoglycan (GAG), which is also an important part of the acellular matrix (ECM). Many active growth factors are tightly bound to specific ECM through heparin HS molecules [6], among which the binding of FGF2 to heparin is the most thoroughly studied.

4 Conclusion

Growth factors have very strict requirements on extraction conditions. Different growth factors have different suitable extraction methods due to their different types and mechanisms of action. This experiment compares the pretreatment methods of SIS material growth factors, and obtains the most suitable extraction methods for three growth factors (TGFβ1, VEGF, FGF2) and detects their contents. The content of TGFβ1 in VIDASISTM is 8375 ± 2125 pg/g, the content of VEGF is 5486 ± 1043 pg/g, and the content of FGF2 is 3990 ± 1372 pg/g; the content of TGFβ1 in

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Biodesign® is 11517 ± 331
The content of pg/g, VEGF is 5432 ± 272 pg/g, and the content of FGF2 is 5417 ± 947 pg/g.

Fund project

This research was supported by the National 863 Program New Materials Special Project (2015AA033602), the Beijing Science and Technology Rising Star Program (Z181100006218013) and the Small and Medium-sized Enterprise Innovation Fund of the Ministry of Science and Technology (Z14010101281).

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DOI: 10.12677/ms.2018.85070