RESEARCH PAPER | Veterinary | Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

Collagen Applications in Biotechnology

KEYWORDS: collagenic granules, microcarriers from untanned collagen-containing leather waste

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ABSTRACT *Collagen is a cell surface transmembrane layer component. Collagen may be used in biotechnology as a microcarrier for culturing of animal cells in viral vaccines production as well as affinity chromatography sorbent material.*

1. Introduction

Collagen known as extracellular matrix glycoproteid is additionally a cell surface transmembrane layer component. It takes an active part in cell adhesion and cell spreading over supporting medium. This creates a potential for using collagen in biotechnology as a microcarrier for quasi-suspension culturing of anchorage-dependent animal cells in viral vaccines production as well as affinity chromatography sorbent material.

2. Results and Discussion

We have developed and patented a production process to extract microcarriers from untanned collagen-containing leather waste. The waste is first converted to the electrically neutral stock collagenous mass with about 20-25 % solids content. The resulting semi-finished product is then electricized and injected under pressure into oil or oil-containing emulsion through metal nozzle. The resulting biphasic mixture is shaked applying relatively low-frequency ultrasound. The product consists of spherical particles of partially denatured collagen bearing the electric charge of the same sign as previously applied. The reagents and conditions utilized to degrease and tan the granules contributed to homogeneous strong cross-linking their internal structure that created a potential for performing sterilization of the semi- finished product via autoclaving without detriment to the shape of the particles. The subsequent incubation of the cross-linked denaturated collagen in the native collagen solution results in inhibition of all unlinked aldehyde groups present at the surface of the particles that finally makes it possible to generate spherical matrix with native collagen molecules coupled to its surface.

The finished product obtained repeatedly according to our technology comprises transparent spherical particles colored yellowish-brown, see Figure 1.

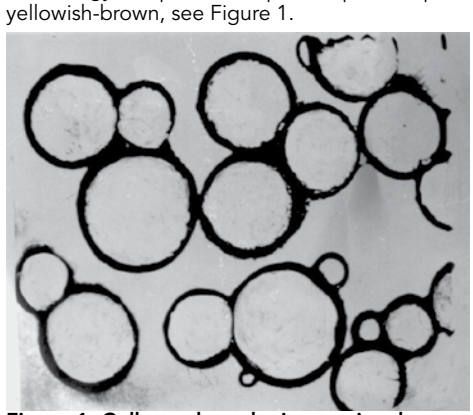

Figure 1: Collagen-based microcarriers by appearance

Table 1 summarizes the experimental results from determining physicochemical characteristics and relevant biological activity over the test batches of collagen-based microcarrier referred to as CYTOCOL.

Table 1: Comparison of physicochemical and biological properties over test batches of collagen-based microcarrier

Batch No.	Particle diameter (10^{-5} m)	Rate of sedimentation (10^{-2} m/s)	Specific gravity (10^3 kgr / m^3)	Total growth surface area per 1 cm^3 of hydrated granules (m^2)	Particle charge sign	Adhesive activity (as percentage of cells attached in 12 hours)	Monolayer formation time (days)	Cell growth multiplication factor (in 5 days)	Cell subinoculation time (days)
1.	10.0	0.21	1.1180	0.19	No charge	70	5-6	3-4	10-12
2.	5.0	0.15	1.1180	0.38	- +	62 81	5-6 2-3	2-3 5-6	15-17
3.	2.0	0.09	1.1180	0.94	- +	64 92	4-5 1-2	2-3 6-7	15-17

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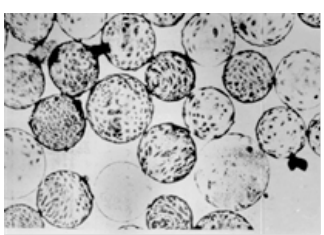
As is seen from the data presented by Table 1, microcarrier particle sedimentation rate increases with particle (granule) size. Specific gravity of granules, on the other side, is the same for particles of any size.

An important characteristic to determine the potential of microcarrier as particle attachment substrate is the total growth surface area per 1 cm^3 of hydrated granules determined by calculation for each batch of microcarrier. It seems evident that the greater the growth surface area, the greater amount of cell mass may be obtained from cultivation. As is seen from the Table, there exists an inverse relation between microcarrier particle size and growth surface area.

When selecting the best microcarrier fraction the fact should be taken into account that the normal cell adhesion and proliferation requires microcarrier particles size at least 100 times greater than the cell size. The potential influence of the microcarrier electric charge upon the cell matter adhesion rate has been studied experimentally as well. It was found that the subinoculation culture cells bearing low negative electric charge are best adherent to the positively charged microcarrier granules with an electrokinetic potential of 7.3 mV to 10.6 mV.

Figure 2 illustrates the results of the experimental testing of Cytocoll microcarrier in the laboratory where the microcarrier was used to cultivate the Taurus-1 subinoculation cell line.



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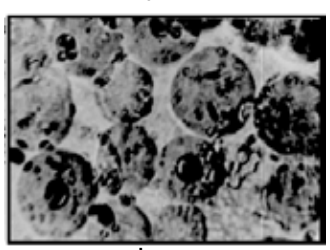

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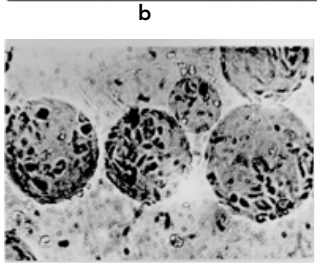

c

Figure 2: Cell monolayer formation dynamics at positively charged microcarrier granules with particle diameter of 20 µm (a), 52 µm (b) and 100 µm (c) after 12 hours of culturing.

As is seen from the Figure 2, the use of positively charged collagen-based microcarriers with particle diameter of 20 µm is in favor of the fully developed Taurus-1 (T-1) cell adhesion to these microcarriers as early as in 12 hours after the start of the experiment.

We also used collagenic granules as affinity chromatography sorbent material to isolate fibronectin from human plasma with due account for its collagen-binding ability as a ligand.

Plasma fibronectin levels are subject to variations within 180 mg/L to 600 mg/L; the total sorption capacity, however, makes it possible to perform exhausting fibronectin extraction, see Table 2.

Table 2: Sorption material characterization in terms of the target product yield

The batch of plasma to be sorbed	Initial plasma fibronectin concentration (mg/L)	The isolated amount of fibronectin	
		mg	%
1	230	225	97.8
2	420	410	97.6
3	305	300	98.3
4	190	187	98.4
5	280	270	96.4

The fibronectin elution diagram below illustrated by Figure 3 demonstrates the isolated preparation purity and homogeneity.

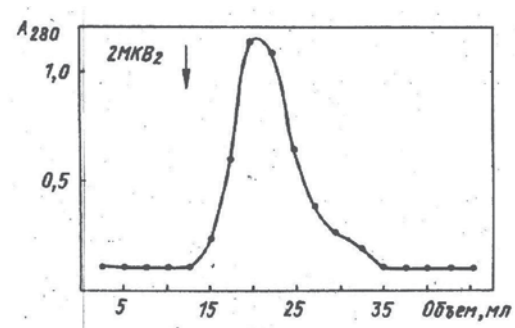

Figure 3: The elution diagram where fibronectin is eluted from collagenic sorbent material

High quality of the target product is further supported by polyacrylamide gel disc electrophoresis (see Figure 4) and immunoelectrophoretic examination (see Figure 5).

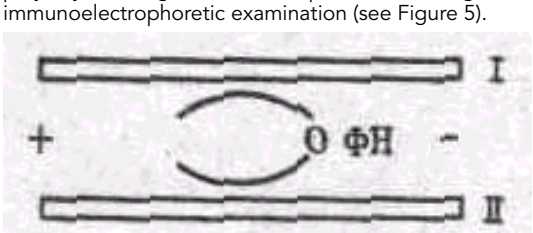

Figure 4: Serological homogeneity of fibronectin during immunoelectrophoretic examination: 1, human plasma protein antiserum; 2, monospecific fibronectin antiserum

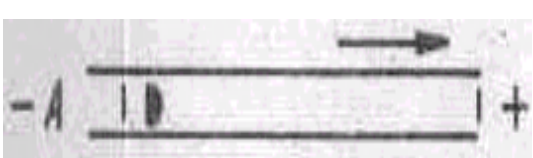

Figure 5: Homogeneity of fibronectin during polyacrylamide gel SDS electrophoresis.

Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

Table 3 summarizes the experimental results of biological activity testing with the fibronectin eluted from collagenic sorbent material considering the indicants such as the fibronectin effect on cell adhesion and cell spreading over the surface of microcarrier.

Table 3: The effect of fibronectin on cell adhesion and cell spreading over the surface of collagenic microcarrier

Microcarrier surface nature	Adhesion (%)	Cell spreading over the surface of microcarrier (%)
Collagen	18	12
Collagen treated with fibronectin	88	47

So, the use of collagenic granules as immunosorbent material creates a potential for producing bioactive fibronectin capable of increasing the cell adhesion by 70 % and cell spreading over the surface of microcarrier by 35 % against control.

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