ACHIEVEMENTS AND LIMITS ON THE OBTAINING OF TEXTILE FABRICS FOR THERAPY OF BURN WOUNDS WITH LARGE SURFACE

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ABSTRACT

Paper carries out the results obtained up to now by performing a blouse and trousers worn by the patient with burn wounds. Each garment is composed by three layers, the outer layer, the next is an intermediate layer and the last is the inner layer. The inner layer is a non-woven structure where a film of a mixture of cellulose and keratin is attached. The inner layer absorbs the exudate from the wound and acts as a dressing; the bicompatibility and in vivo tests are presented. The outer layer is a woven fabric made by 100% cotton.

Key Words: layers, keratin, bacterial cellulose, burns

1. INTRODUCTION

The patients with large surface burns represent an acute problem and their surviving chance depends on the complex action of medical assistance. The most critical period is the first week, when the painful shock, the deficit of skin and liquids and microbial invasion respectively need to be solved. The presence of the costume is useful in so far as it simplifies the medical procedure. Any textile solution that complicates the procedure is unpractical. A textile garment must solve the condition of sterility, of favouring the wound re-epithelization conditions and electrolytes transfer to the dermis.

The purpose of the work is to obtain a textile assembly in two or more variants: i) a medical pajamas consisting of a blouse and trousers; ii) a medical vest. The choice of one of the other possibility is decided by the clinician. Regardless the option, the textile structure contains three layers, as illustrated in figure 1.

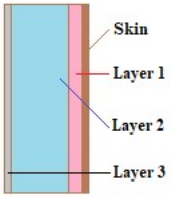

Figure 1. Scheme of layers setup versus skin

In the image from figure 1, the layer 1 is the first layer in the proximity of the burn and it is destined to facilitates the re- epithelization, no matter whether the pajamas is worn immediately after patient admision to hospital, after wound debridling or after skin grafting.

Layer 2 provides the necessary electrolytes by diffusing the electrolytes to the skin, and it contains a pain-killer and silver sulfadiazine as anti-microbial agent. Layer 3 plays the role of thermal protection of the body, because the patient with a significant lack of skin looses body heat, having hypothermal thrills regardless the external temperature. The work presents the stage of research concerning the construction, biocompatibility and biological behaviour of Layer 1.

2. GENERAL CONDITION OF THE PATIENT WITH BURNED WOUNDS

The patient with burns arrives in the clinic and is evaluated medically. He is in an acute painful schock, and extending the lapse of time from the place of accident to clinic makes the patient condition worse. Skin destruction through burning means loosing electrolytes and enzimes, with dramatic affections at circulatory, digestive and renal levels, as well as of the nervous and breathing systems, with specific manifestations due to the proliferation of pathogen agents, blood viscosity modification, and a very bad general condition. Figure 2 illustrates the image of a patient with burnt upper limbs.

Figure 2. 4th degree burn infected with Pseudomonas aeruginosa

3. PRODUCTION OF LAYER 1

Layer 1 consists in keratin hydrogel, bacterial cellulose and a biologic tissue obtained from stem cells by tissue engineering. A series of arguments decided the choice of human keratin as basic polymer for biomaterial construction. These, with the condition to be accepted in the biologic medium and with performances of cutaneous regenesis through plastic re-formation. The safety of the choice is based on an information from Chinese traditional medicine which, through a millenary experience, has verified the therapeutic potential of keratin utilization as a remedy for burns. This implies sampling the hair that, by burning, becomes an opaque dark colour mass which then, through milling, results in a keratin-based powder. The powder is spreaded over the burn, this procedure being in China a popular wound therapy, verified over about 1300 years [1]. Other arguments led to the use of the advantages due to bacterial cellulose, as being the second constituent of the basic biomaterial, which is based on the nanometer size of cellulose filaments, their good strength and chemical purity.

3.1 HYDROGEL PRODUCTION FROM KERATIN

The hydrogel preparation from keratin consists in the following procedure:
One collects by cutting at 2- 5 cm length, undyed capillary bristles, from men, regardless the age, from a hairdressing saloon. Then, the collected hair is cleaned by removing the lipids in a Soxhlet, for 24 hours, using a $CHCl_3$ and CH_3OH mixture in a ratio of 2:1 (v/v). The hair is dried at 40°C overnight. The cleaned hair is introduced with a quantity of distilled water in a sealed capsule made of stainless steel, at a solid:liquid ratio of 2:35 (w/w). The capsule is introduced in an air-heated drier at 150°C for 2 hours. Inside the capsule, a water vapor pressure of 4.9 atm (about $48 \cdot 10^4$ Pa) is developed, according to Pavlov [2]. After treatment, one separates through centrifugation for 10 minutes at 10,000 rot/min, two phases: a solid, water-insoluble phase, considered as beta-keratose, and a liquid, water-soluble phase, namely gamma- keratose.

In order to prepare the beta-keratose film, the material is subjected to an oxidation treatment for 3 hours at boiling, using 4 g solid phase and an oxidation agent (30%), at a ratio of 1:4 (w/v). The obtained solid phase is filtered and then washed 6 times with 100 ml distilled water each time; then it is dried for 24 hours at 40° C. Then, in beta-keratose emulsion, one adds a reducing agent in six equal portions of 0.3g each, at 1 hour difference, at cold. This treatment is followed by solution settling on bacterial cellulose and lyophilization.

The bacterial cellulose is obtained in the form of a nanofibres veil by seeding Acetobacter xylium in a culture medium at 37°C for two weeks. Bacterial cellulose has very advanced fineness, as compared to other fibrous polymers, at the order of tens of nanometers; it is more crystalline than usual cellulose obtained through classical technologies for cellulose processing from paper or textile industries, and it is a chemically pure product.

3.2 BACTERIAL CELLULOSE PRODUCTION

According to some bibliographical sources [3, 4], cellulose synthesis by Acetobacter xylinum occurs following two processes, a polymerization one, and a crystallization one, both catalyzed by enzymes. It has been computed that an Acetobacter xylinum cell develops a polymerization speed of up to 200,000 glucose molecules/sec. [3, 5]. Between BC and the bacteria that synthesize it there is a symbiotic relationship. It is considered that bacteria synthesize the cellulose because the last one retains an increased amount of water, which prevents bacterium dehydration, ensuring its vitality for a longer time interval.

Cellulose also captures carbon dioxide molecules resulted from cellulose synthesis, ensuring for bacterium a good capacity to float over water surface, having a facile access to the oxygen from air; as bacterium is anaerobic, this means a good ability to feed at the interface with atmospheric air. Due to its opaque nature, cellulose film protects the bacterium against the destructive and dangerous effect of UV radiation [6]. Alternatively, by cellulose dissolution in water, a barrier is formed against medium colonization by other bacteria that would compete with the host bacterium. Two procedures were used for bacterial growth.
Procedure 1
Yeast medium with nutrients: Yeast extract 5.0 g; Peptone 3.0 g; Mannitol 25.0 g; Distilled water 1000.0 ml. pH is not adjusted. One adds agar at a concentration of 12.0 g/l.
Procedure 2 Oxidation medium of Acetobacter xylinum microorganisms: Glucose, 100.0 g; Yeast extract, 10.0 g; $CaCO_3$, 20.0 g. Distilled water 1000.0 ml. pH= 6.8. $CaCO_3$ in week acid

medium serves as buffer. Calcium carbonate is placed in agar plates before agar gets stabilized, producing an opaque layer on recipient bottom. As microbial stem increases and acid is produced, this reacts with calcium carbonate, determining it to get solved and form specific cleaning zones just under the formed colonies. In liquid medium, calcium carbonate also forms sediment that can be cleaned with an acid product.

3.3 BIOLOGIC TISSUE PRODUCTION

After mixing the hydrogel with bacterial cellulose, one obtains a film of gelatinous consistence. The film is subjected to lyophilization, sterilization and to sowing with stem cells resulted from the adipose tissue of a rabbit. One obtains a biologic tissue on a structure of keratin and bacterial cellulose. Biocompatibility tests were carried out then, to determine tissue biological performances.

The direct contact method was used to evaluate the cellular biocompatibility. Sterilization of the materials that are to be analyzed is a very important aspect, representing the first step in tissue realization. In view of sterilization, in the first stage fragments of the materials to be tested were exposed to UV radiations for one hour. The procedure has continued with immersing the exposed fragments in sterile alcohol 70% for 30 min., washing with sterile distilled water for 30 minutes more, and then the fragments were immersed in HBSS (buffer solution) for other 30 minutes, a stage repeated three times. Last stage consisted in the immersion in DMEM culture medium (4500 mg/L glucose, L-glutamine, and sodium bicarbonate, liquid, sterile- filtered) 3 hours before tissue realization.

The stem cells are sown in the culture medium, DMEM + 10% BFS (calf fetal serum) + 1% P/S/N (a mixture of penicilin – streptomycin – neomycin) and incubated for 24 hours (5% CO_2, 37^{o}C). After 24 hours, the culture medium is changed and material fragments were brought in straight contact with the cells. Culture plates with 48 buckets were used.

The MTT (3-(4,5-Dimethylthiazol-2–yl)-2,5 Diphenyltetrazolium Bromide) test was performed after 24h, 48 h and 72 h respectively of direct contact of materials with cells. In order to perform the test, the culture medium from buckets was replaced with MTT solution (5%). The cells with MTT solution were incubated at 37^{o}C for 3 h. The viable cells reduce the MTT tetrazolium solution to a coloured product named formazan. The formazan is solubilized with isopropanol. The absorbance of the resulted formazan solution was measured spectrophotometrically al λ= 570 nm with a Tecan Sun-Rise Plate Reader. The results of spectrophotometric reading from the experimental buckets have been reported to the control buckets with no material present, and the cell viability (V) was computed:

$$\mathbf{V} = \frac{abs}{abs\ control} \times 100 \quad (1)$$

Where: *abs* is absorbance from the buckets with materials, and *abs control* is the control absorbance.

Cell viability tests show that after 24 h and 48 h respectively after sowing, in the case of gamma keratin (soluble fraction) there is a diminution of the cell number from 100 to 95 and 98 cells respectively, and after 72 h, there is an increase to values of 112% cells. In the case of beta keratin (insoluble reticulated fraction) manifests a cell viability of 107, 119 and 117% respectively after 24, 48 and 72 hours. In the both cases, the cell viability after 72 h. shows that, on the considered biomaterials, one obtains a biologically active fraction, with a higher

potential in the case of beta keratin. One has registered the appearance of cell growth buds and the development of a tissue.

3.4 EXPERIMENTS ON ANIMALS

Rabbits of common domestic breed were used to inflict two burnt wounds under anaesthetic, after which, one grafted on one burnt wound a biomaterial consisting of bacterial cellulose and keratin hydrogel, and on the second burnt wound - a biomaterial of bacterial cellulose and keratin, plus a tissue consisting of stem cells taken from rabbit adipose tissue. After grafting, the animals were supervised for a period of up to three weeks. The wound aspect is illustrated on the micrographs presented herein after.

Zone delimitation. Preparation of the back zone by shearing and delimitating with a marker a surface of 3 x 3 cm. Xylin was injected hypodermally in four points for local anesthesia and diminution of post-burn inflammation.

Zone hygienization. Derm preparation is performed by desinfecting the surface with Betadine. The motions of impregnation with iodine are circular, from the middle to the exterior.

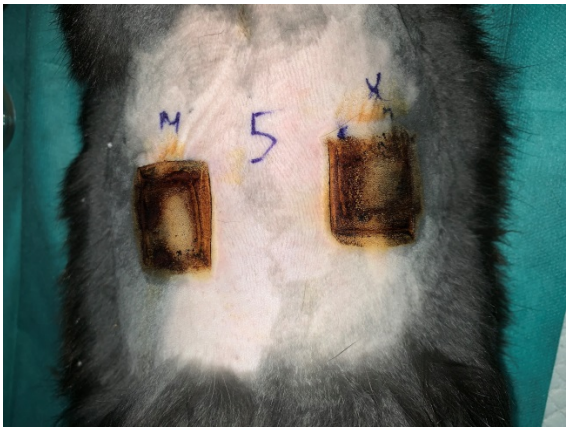

Figure 3. Burn in itself

The two burns, one marked with M for biomaterials of BC and keratin hydrogel, and the other marked with X with biomaterial of BC, keratin hydrogel and tissue (rabbit stem cells).

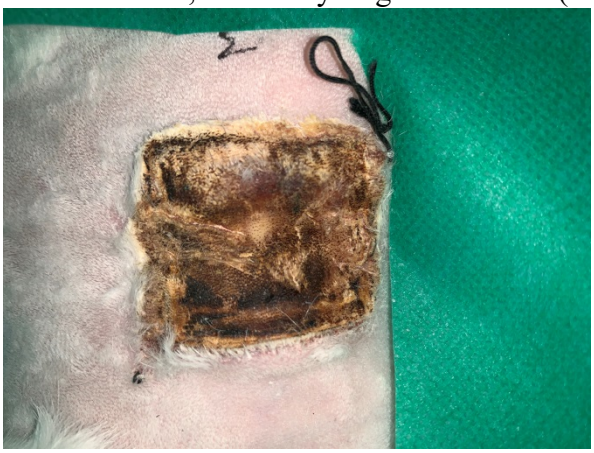

Figure 4. Sampling 14 days, graft M without tissue

One can notice as obvious bristle reappearance along the burn contour, lack of inflammation and, after taking biologic samples for anatomo-pathologic tests, tissue re-vascularization. At palpation, the zone presents a hard, normal aspect, without pain; there is, yet, the same itchiness tendency.

4. CONCLUSIONS

A biomaterial was obtained from bacterial cellulose, through the activity of Acetobacter xylinum microorganisms and from keratin derivatives. On this biomaterial, stem cells from rabbit adipose tissue were sown for tissue growth. The biomaterial was grafted on the burn induced in an *in vivo* study on common breed rabbits. The experimental protocol permits to obtain either an artificial tegument used as skin for grafting, or a biomaterial that should facilitate the classical clinical procedure of self-grafting from the very patient. For layers 2 and 3 of the textile suit, the research is in progress.

5. REFERENCES

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