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A. **Braidot**, A. **Hadad** (Eds.)

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This volume presents the proceedings of the CLAIB 2014, held in Paraná, Entre Ríos, Argentina 29, 30 & 31 October 2014. The proceedings, presented by the Regional Council of Biomedical Engineering for Latin America (CORAL) offer research findings, experiences and activities between institutions and universities to develop Bioengineering, Biomedical Engineering and related sciences.

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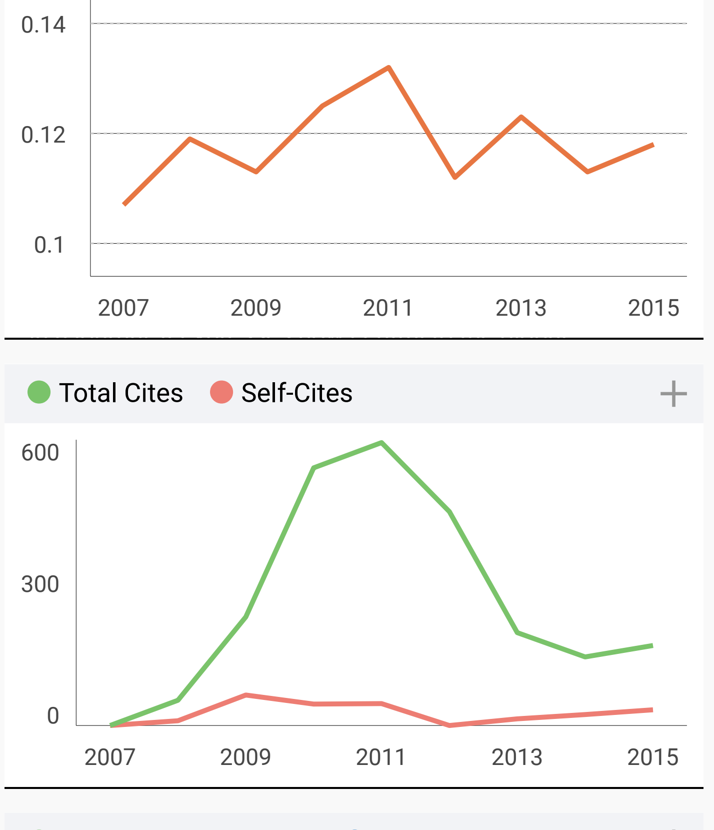


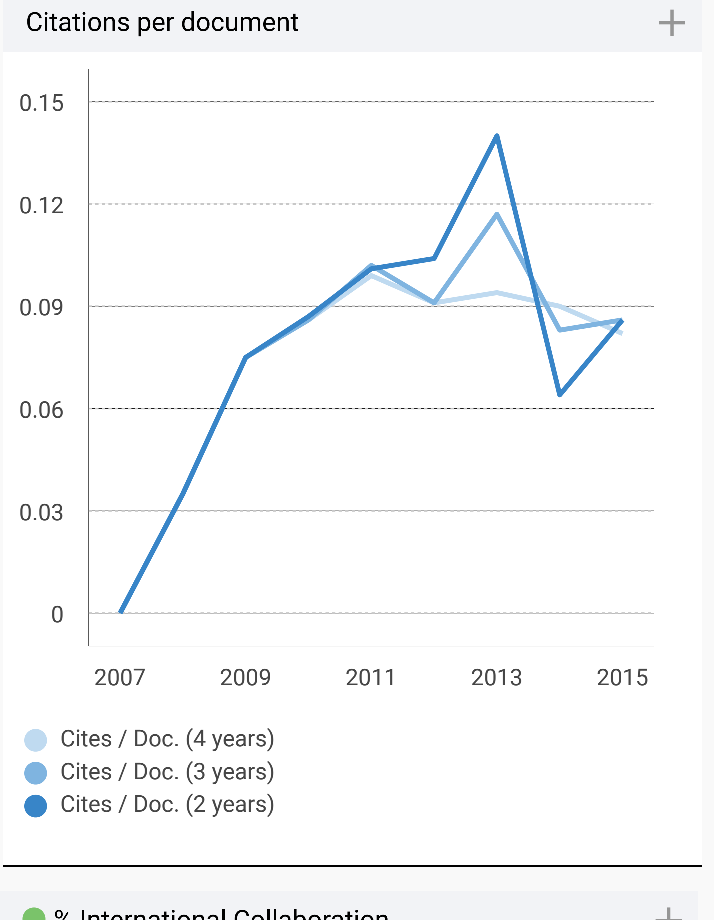


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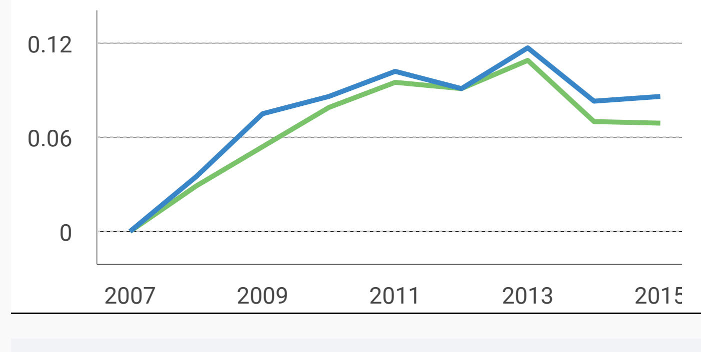


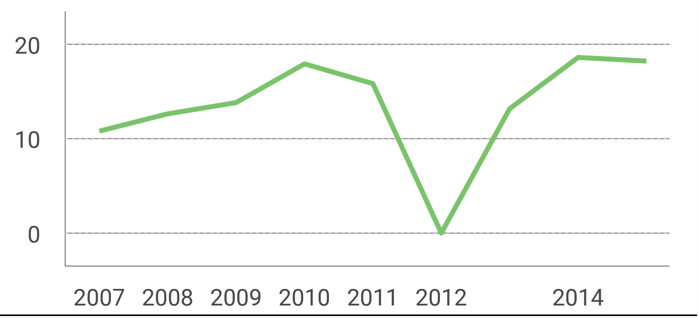


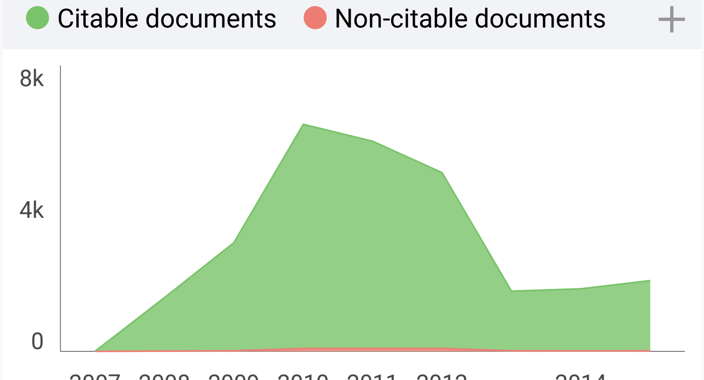


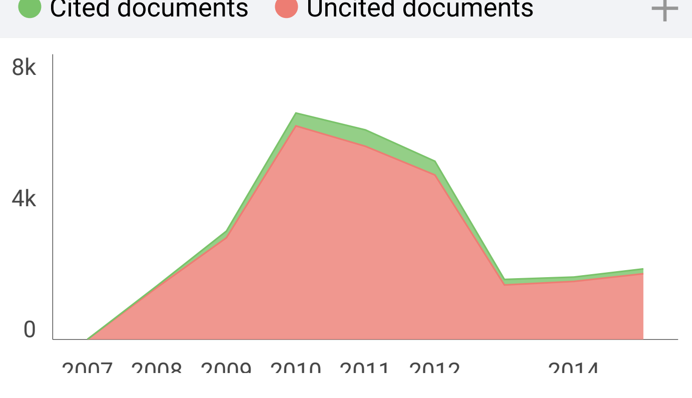


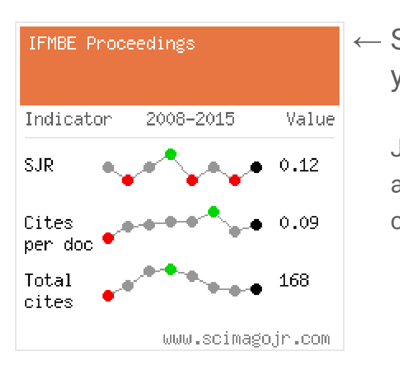


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# Keratinocytes Growth Using Polyvinylidenefluoride (PVDF) Fiber Mats by Electrospun

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*Abstract*— **There is a growing demand for skin transplants; however, immunological incompatibility to such transplants remains as an unsolved issue. It is necessary to improve the current production methods for epidermal originated tissue. PVDF-based membranes have the potential to be used as an appropriate scaffold for skin cell growth. There are a wide range of medical applications and potential medical applications of PVDF. Keratinocytes on PVDF scaffolds had the greatest alkaline phosphatase activity and early mineralization. The results demonstrate the potential for the use of PVDF scaffolds for keratinocytes growth applications.)**

*Keywords*— **Fiber mats, Scaffolds, electrospinning, keratinocytes, PVDF..**

## I. Introduction

(Keratinocytes conform the majority of cells in the epidermis. Moreover, they are one of the few animal cells that can grow in any type of cell growth culture: explant culture, dissociated cell culture, three-dimensional culture and cell line culture. There is a lot of research to be done in order to improve the production of skin transplants for a faster healing. Specifically, the creation of a (base) membrane to facilitate the in vitro formation of a complete epithelium is of great interest [1].

In recent years, there have been many keratinocyte techniques developed for therapeutic purposes, from which sheeting of epidermis can be achieved in a relatively short period, usually between 20 to 22 days. A breakthrough for obtaining epidermal crop was the use of epidermal growth factor (EGF), which resulted as a potent stimulator of cell growth in vitro, however, it is until 1981 when O 'Connor achieved to make a in vitro human skin graft obtained from the same patient [2].

The widespread implantation of alloplastic materials in muscles and facial tissue is known to cause complications such as restriction of mobility of the abdominal wall, induction of intra-abdominal adhesions with erosion of adjacent organs, and so on. To avoid this problem the current mesh prostheses are made from polypropylene (PP), polyethylene terephthalate (PET) or polytetrafluoroethylene (PTFE), although these also have some disadvantages.

Polyvinylidene fluoride (PVDF) is a polymer with better biological and textile properties. In comparison with PET, PVDF is more resistant to hydrolysis and degradation, in addition, the aging of the material does not increase its rigidity which occurs with polypropylene. Even though it has been introduced in vascular surgery years ago, it has never been used for the construction of surgical meshes [3]·

## II. Methodology

*Preparation of the skin sample from Rattusnorvegicus:* The keratinocytes were obtained after the rat was sacrificed following the according ethical considerations. Before taking the skin sample, the rat was carefully shaved and cleaned with an alcohol solution at 70%. Skin samples were taken, later on, they were cleaned of phosphate-buffered saline (PBS) under sterile conditions. Once the skin ex- plant was clean, the tissue was cut into small pieces on top of a glass microscope slide.

*Dissociation of skin cells:* Once prepared, the sample was placed on a Falcon tube containing a solution of 17 mL of PBS, 2 mL of G-Penicillin and Streptomycin. 4 mL of Trypsin were added and the enzyme was left working during 20 minutes at 27º C, while homogenizing within a laboratory pipette.

Keratinocyte Purification: Homogeneous solution was centrifuged at 200 x g for 5 minutes, the supernatant liquid was discarded and the cell pellet was conserved.

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*Keratinocyte Culture:* The cell pellet was placed and uniformly distributed on the PVDF membrane on the Petri dishes, special care was taken on placing the cell pellet exclusively on top of the PVDF membrane. The cell pellet was resuspended in 4 mL of DMEM (Dulbecco's Modified Eagle's Medium) media enriched with Fetal Bovine Serum. Finally, cells were left to grow on the incubator under the following conditions: 37º C, 5% of $CO_2$ content on the atmosphere, and 100% of relative humidity. Cells were observed on a daily basis through the inverted microscope (Olympus IX 71). After five days, the cells were provided with fresh media. After 12 days the cells were stained with blue methylene in order to check cell viability.

*Staining with blue methylene:* PVDF membrane was stained with blue methylene. This kind of staining allowed us to confirm the presence of keratinocytes through the microscope.

*PVDF membrane:* Electrospinning technique was used to produce the membrane. This technique is based on using electric forces to form fibers from liquid polymers. It does not require high temperature yields or chemical reactions in order to transform a solid from a liquid solution. Very thin fibers (<100 nm) can be obtained from great molecular weight compounds. When a liquid polymer is transformed to fibers with electrospinning, many characteristic shapes of the material can be found. This method of obtaining thin fibers can be used in many different ways, from making biomedical devices to solar panels. The electric charge causes a beam to emerge from a so-called Taylor cone. The charged beam from the material (in this case PVDF) first elongates towards a recollector in a straight line to then bend in a spiral shape. The repulsive force between charges in the beam causes it to elongate and then become thinner. This elongating and thinning of the beam is the one that causes the fiber solidification [4]. PVDF membranes were prepared using 10 PVDF pellets of 0.06 gr each, dissolved in 2.4 gr of solvent (N, N Dimethylformamide (DMF) in order to obtain concentration of 20%.

Membrane Sterilization: PVDF membranes were sterilized using UV light for 2 hours in the laminar flow hood to UV light in laminar flow cabinet (ESCO). A sterilization method based on UV light was chosen in order to leave the structural and functional characteristics of the membrane unaffected. After being sterilized, one membrane was placed on a plastic Petri dish, while the remaining membrane was placed on a Petri dish made of glass.

## III. RESULTS

After five days of growth in the incubator, and before providing the cells with new media, cell development was captured on photographs. Different areas of the membrane were captured in order of detect possible cell growth and a possible membrane degradation as shown in Fig. 1.

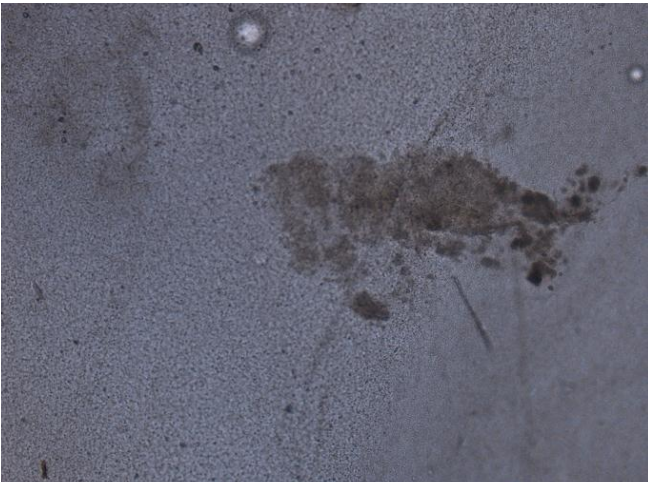

Fig. 1. Agglomerates observed on the microscope after 5 days of growth.

In Fig. 2, After 12 days of growth, a viability test was performed through a blue methylene staining technique; once again, different areas of the membrane were examined for cell growth and possible membrane degradation.

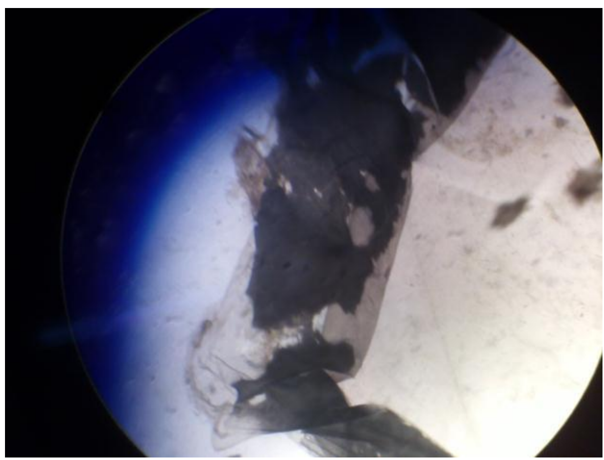

Fig. 2. Close up to the stained PVDF membrane after 12 days of growth

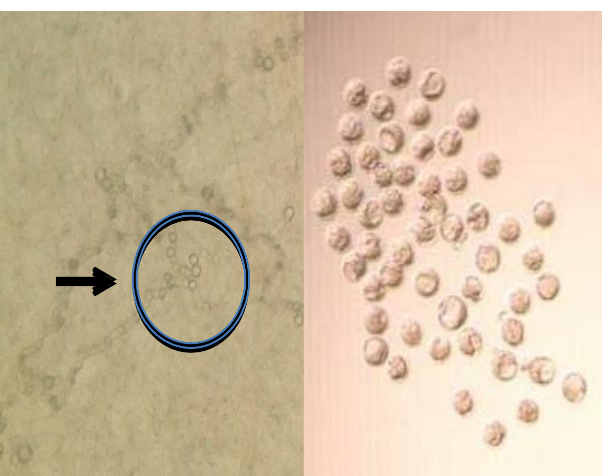

Fig. 3. Close up to keratinocytes after 12 days of growth (left) and purified mouse keratinocytes from an embryo (right) [5].

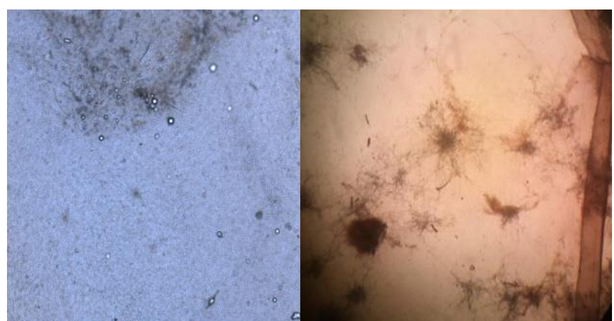

Fig. 4. Comparison between close ups of the PVDF membrane after a 5 day growth (left) and 12 days of growth and a blue methylene staining (right).

Fig. 5. Comparison between the state of the PVDF mem- brane before the experiments (left), after 5 days of cellgrowth (center) and after 12 days of cell growth and a blue methylene staining (right).

## IV. DISCUSSION

These experiments were done in order to evaluate the capacity of a PVDF membrane to serve as a scaffold for skin tissue development. Presence of multiple agglomerates was observed on the microscope after 5 days of cell culture as observed on Fig. 1. Although presence of cells can be suspected through observance of such agglomerates, it is impossible to determine cellular growth using agglomerate formation as a sole indicator. Such dark spots on the microscope could be the consequence of a cell aggregate of keratinocytes, other cell types, a biological contamination or a possible non uniform conformation of the PVDF membrane.

In order to generate a comparison between the dark formations of unidentified source, observed on the microscope, and the dark formations caused by agglomerates of PVDF membrane; a close up photograph of a dark formation area was taken , it became evident that the source was a PVDF membrane agglomerate.

Because of the impossibility to determine rat keratinocyte cellular growth on the PVDF membrane after just 5 days of culture, the culture time was extended to 12 days. Unfortunately the culture growing on the glass Petri dish suffered a biological contamination, therefore the culture was discarded. However the culture on the plastic Petri dish remained uncontaminated, and after 7 days, a staining with blue methylene was done.

Fig. 2 shows an agglomerate of PVDF membrane after the blue methylene staining. Fig. 3 shows a comparison between a close up to Fig. 2 and a reported image of Keratinocyte isolation [5]. Similar morphological characteristics can be observed between cells of both pictures, which points out to a possibility of the PVDF membrane to sustain rat keratinocyte cells. However confirmation tests are to be done. Immunohistochemical tests with confirmed keratinocyte markers are to be done in order to confirm keratinocyte presence.

Biodegradation is very important if PVDF is to be used as a scaffold for skin tissue culture along with the capacity to generate adherence and sustaining cellular growth. Such a characteristic can be hinted on Fig. 4, where a close up to the membrane is done, after the cell culture.

While observing Fig. 5, a change on the consistence of the PVDF membrane is observed, this seems to support a possible biodegradation of the membrane as a direct result of cellular growth. However no affirmation of such ability can be done without further tests.

## V.CONCLUSIONS

There seems to be certain indications that the produced PVDF membrane fibers are capable of sustaining rat keratinocyte growth and such growth could be the source of a hinted possible biodegradation of the PVDF membrane. Such characteristics would make the produced PVDF membrane as a good candidate as a scaffold for skin tissue.

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