Composite PLLA/PP Monofilament 4DVentral® Promotes an Anti-Inflammatory Response In Human Macrophages *In Vitro*.

Mehdi Daoudi[1*], Cynthia CALLIGARO [2], Nihal Engin VRANA[2], Madjid Tagzirt[3*] and Michel Caillibotte[1]

*Corresponding authors: *m.daoudi@cousin-surgery.com*

[1] **COUSIN SURGERY** (Biotech), ALLEE des Roses, F-59117 Wervicq-Sud, France.
[2] SPARTHA MEDICAL, 1 Rue Eugène Boeckel, F-67000 Strasbourg, France
[3] Univ. Lille, Inserm U1011- European Genomic Institute for Diabetes (EGID), Institut Pasteur de Lille, F-59000 Lille, France.

BACKGROUND AND OBJECTIVES

The use of prosthetic mesh for abdominal wall repairs can significantly reduce the risk of hernia recurrence[1]. However, macrophage (MØ) phenotype was reported as a predictor of constructive remodeling of abdominal connective tissue following the implantation of hernia meshs[2]. Inflammation response characterized by an uncontrolled release of pro-inflammatory M1 cytokines or chemokines, and tissue-degrading enzymes has been shown to be responsible for postoperative complications. In contrast, the angiogenic and tissue remodeling activities of the alternative anti-inflammatory M2 pro-remodeling MØ have potential use in tissue regenerative. A new type of hernia implants combines monofilaments, Polypropylene (PP) and a biobased and natural slowly bioabsorbable polymer, Poly-L-Lactic Acid (PLLA). The partially (60%) absorbable PLLA/PP and non absorbable PP meshes manufactured by COUSIN SURGERY are 4DVentral® and Biomesh P1®, respectively. The contribution of PLLA in regulated inflammatory response and in enhancing abdominal repair remains unknown or unclear.

In this present study, we aimed to characterized the inflammatory profiles of macrophages *in vitro* in presence of composite partially absorbable PLLA/PP 4DVentral® compared to permanent PP Biomesh P1® by quantify cytokine release in culture supernatants and mRNA expression levels in human MØs.

MATERIAL AND METHODS

Mesh. PLLA/PP 4DVentral® vs. PP Biomesh P1® meshs.

Sterilization of Mesh. ETO-sterilized.

In vitro assay Human monocytic leukemia cell line. THP-1 (10,000 cells/well in 24-well plate) were differentiated to MØ after stimulation with PMA 100 ng/ml for 3 days. The PMA-differentiated THP-1 MØ (adherent form) were then cultured with or without the meshs and pro-inflammatory cytokines (**Figure 1**).

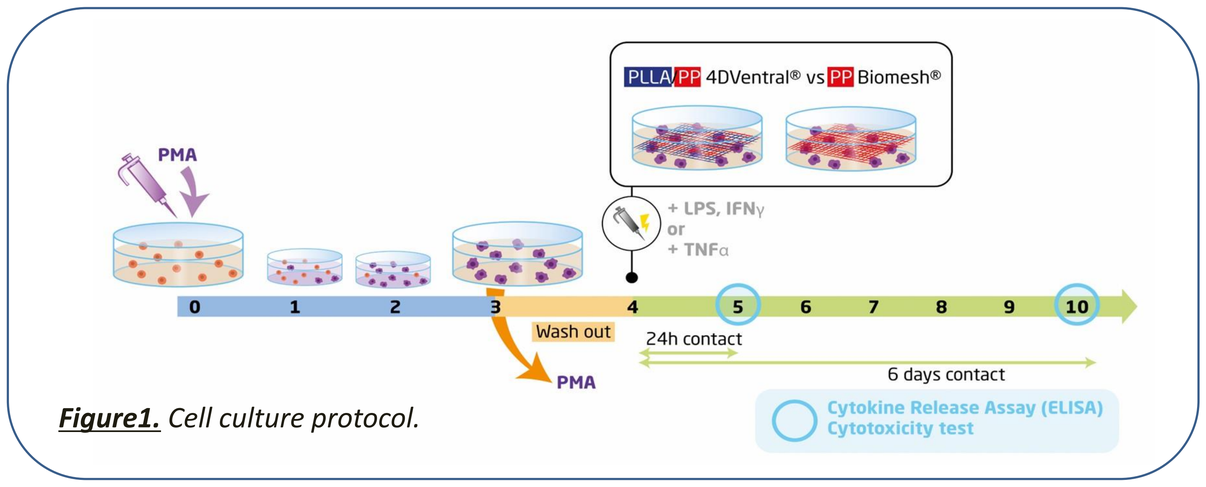


Figure1. *Cell culture protocol.*

Isolation of human peripheral blood monocytes and differentiation. Human blood-derived monocyte isolated by density centrifugation of heparinized blood on Ficoll-Paque were cultured with fresh complete medium (RPMI 1640) containing recombinant human IL-1β (15 ng/ml) to obtain M1 macrophages or IL-4 (15 ng/ml) to obtain M2 macrophages.

Cytotoxicity assay. Cytotoxicity of meshs was evaluated according to the ISO 10993-5 standard. The AlamarBlue® cell viability assay reagent was used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity in direct or indirect (24h mesh-conditioned media) contact with Mesh. *

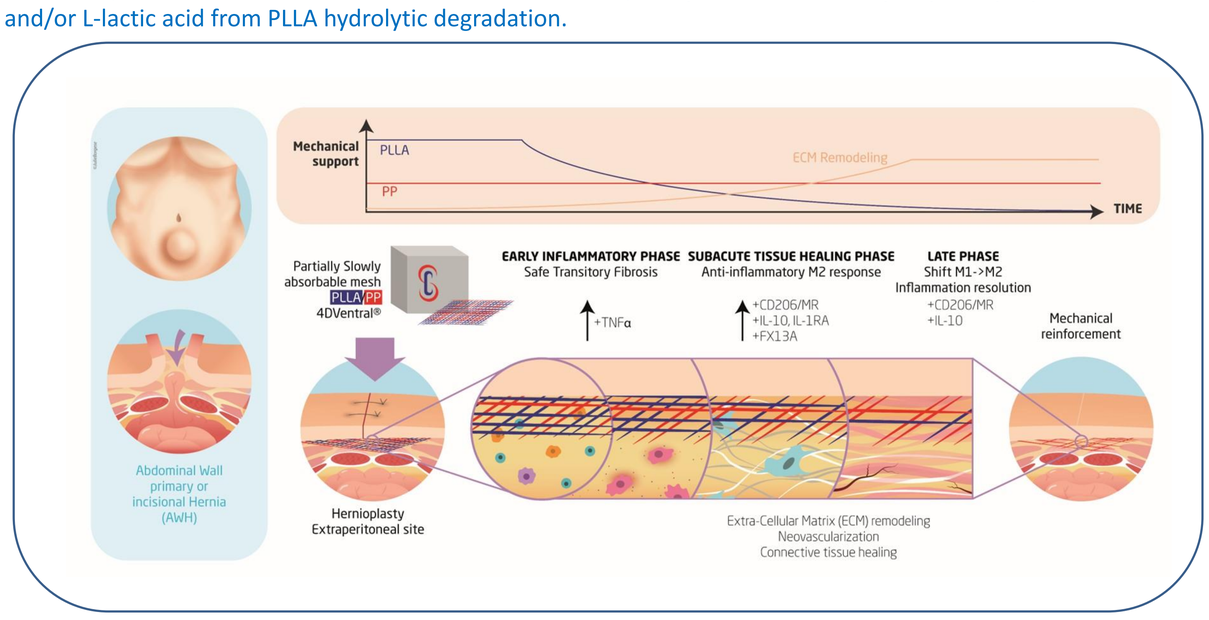
RNA extraction and analysis : Cells were homogenized in TRIzol® reagent. Total RNA was extracted using the chloroform/isopropanol/ethanol method. The gene expression were evaluated by TR-qPCR

Cytokine secretion : cytokines were quantified by Human TNF-α , IL-10 and IL-1R assay kits (Invitrogen, Carlsbad, CA).

DISCUSSION

We found that PLLA/PP 4DVentral® enhances polarization of human naïve MØ to an M2-like phenotype at early time (1-6 days) by increased mRNA expression levels and release of anti-inflammatory markers and cytokines. Interestingly, PLLA/PP 4DVentral® shifted the M1 MØ to an M2-like phenotype, mainly through induction of IL-10 secretion.

Our results shown that composite partially absorbable PLLA/PP 4DVentral® modulates the inflammatory profile of human MØ. The controlled transition from M1 to M2 MØ may be due to the hydrophilic properties of PLLA and/or L-lactic acid from PLLA hydrolytic degradation.



CONCLUSION

It has been reported that with the transformation of MØ phenotype, the increase of IL-10 levels represents the transition of muscle regeneration from proliferation to differentiation and growth [4]. The Composite PLLA/PP partially absorbable **4DVentral®** could promote an anti-Inflammatory response of MØ leading to **Initiate the pro-regenerative process** of defects of the anterior abdominal wall during hernia repair by **targeting the Extra-Cellular Matrix (EMC) homeostasis** of abdominal connective tissue.

While waiting to confirm these results in an experimental model, we highlight the interest of using 4DVentral® for treating patients with wound-healing pathologies restricting during hernia repair, with a guarantee to limit the late-stage hernia recurrence by the presence of the non-absorbable PP to abdominal reinforcement.

RESULTS

1. Composite PLLA/PP 4DVentral® and PP Biomesh P1® meshs are safe on naive THP-1 MØ

The cytotoxicity evaluation was investigated and show that both composite PLLA/PP 4DVentral® and non-absorbable PP Biomesh P1® are nontoxic *in vitro* toward PMA-differentiated naïve THP-1 MØ in direct culture condition (**Figure 2**). Therefore, the values of cytokines produced by these cells and measured from supernatant's are systematically related to the raw values (UA) of the metabolic activity measured in each condition.

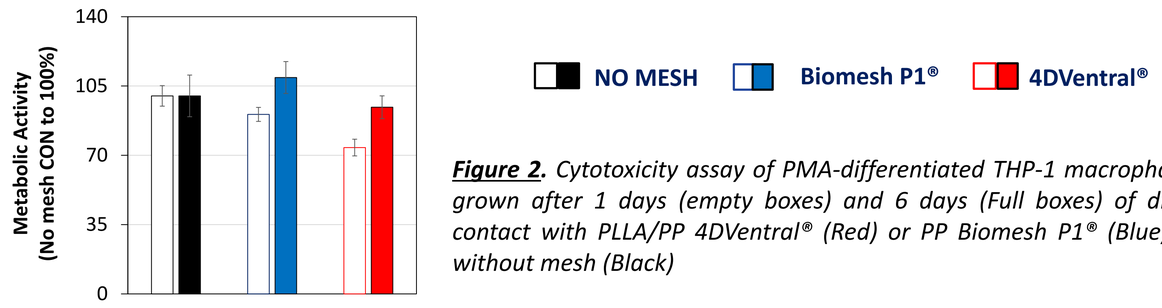


Figure 2*. Cytotoxicity assay of PMA-differentiated THP-1 macrophages grown after 1 days (empty boxes) and 6 days (Full boxes) of direct contact with PLLA/PP 4DVentral® (Red) or PP Biomesh P1® (Blue) vs. without mesh (Black)*

2. Composite PLLA/PP 4DVentral® mesh induces an increased secretion level of anti-inflammatory M2 (IL-10 and IL-1RA) cytokines in naive THP-1 MØ

The composite PLLA/PP 4DVentral® (red histograms) increases the anti-inflammatory IL-10 release after 24h of culture (**Figure 3.A**) and potentiates IL-1RA secretion at 6 days (**Figure 3.B**) in PMA-differentiated THP-1 naive MØ compared to PP Biomesh P1® and no mesh (red and black histograms, respectively)

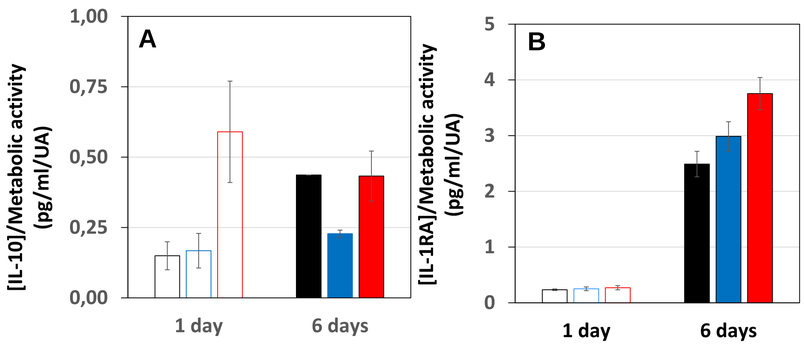


Figure 3*. Anti-inflammatory cytokine IL-10 (A) and IL-1RA (B) levels in the supernatant from of THP-1 naïve (M0) MØ grown after 1 days (empty boxes) and 6 days (Full boxes) of direct contact with PLLA/PP 4DVentral® (Red) or PP Biomesh P1® (Blue) vs. without mesh (Black)*

3. Composite PLLA/PP 4DVentral® mesh increases secretion levels of the pro-inflammatory cytokine TNFα in naive THP-1 MØ

According to the literature about fully resorbable P4HB monofilament mesh (Phasix®, Bard)[3], we found that the composite PLLA/PP 4D Ventral® increases also pro-inflammatory cytokine TNFα release in naïve THP-1 MØ (**Figure 4**) .

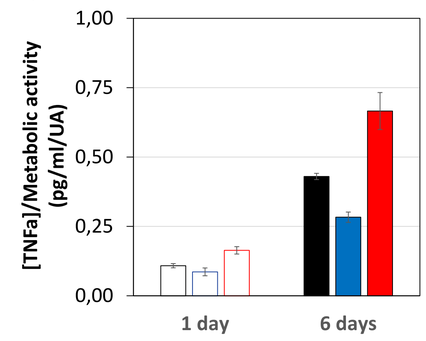


Figure 4*. Cytokine TNFa levels in the supernatant of PMA-differentiated naive THP-1 MØ grown after 1 days (empty boxes) and 6 days (Full boxes) of direct contact with PLLA/PP 4DVentral® (Red) or PP Biomesh P1® (Blue) meshs vs. without mesh (Black).*

4. Composite PLLA/PP 4DVentral® mesh drives pre-activated THP-$1_{INFγ/LPS}$ M1-like to anti-inflammatory M2-Like MØ

The composite PLLA/PP 4DVentral® (red histograms) inhibits the pro-inflammatory IL-6 release (Figure 5.A) and in the same way increases IL-10 secretion (Figure 5.B) compared to PP Biomesh P1® and no mesh (red and black histograms, respectively) in THP-1-derived MØ that were polarized with IFN-γ/LPS to the M1-type both at 24h and 6 days of culture.

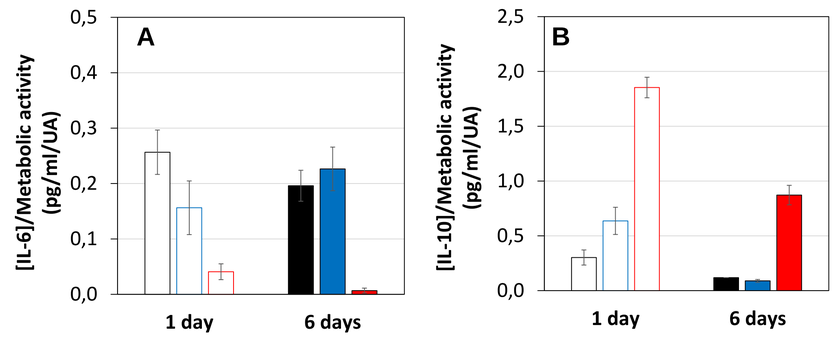


Figure 5*. IL-6 (A) and IL-10 (B) cytokine amounts were higher in the supernatants from of INF$_{\gamma}$/LPS-induced M1-Like THP-1 MØ after 1 days (empty boxes) and 6 days (Full boxes) of direct contact with PLLA/PP 4DVentral® (Red) compared to PP Biomesh P1® (Blue) vs. without mesh (Black)*

5. Composite PLLA/PP 4DVentral® mesh potentiates the anti-inflammatory M2 profile of hMDM

The composite PLLA/PP 4DVentral® (red histograms) potentiates the anti-inflammatory CD206 (**Figure 6.A)** and F13A (**Figure 6.B**) gene levels in IL-4-induced polarization of hMDM compared to PP Biomesh P1® and no mesh (red and black histograms, respectively)

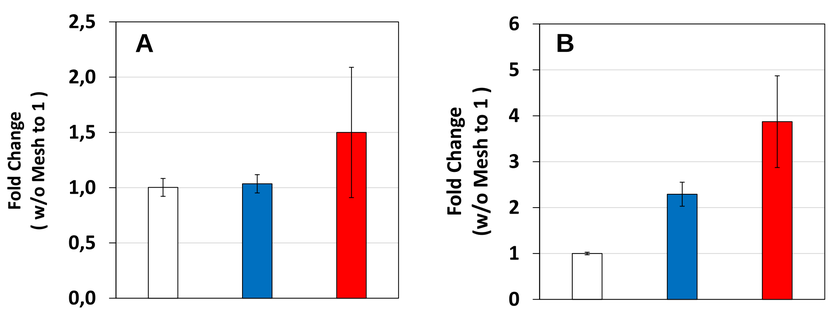


Figure 6*. PLLA/PP 4DVentral® (red) increases the anti-inflammatory CD206 (A) and F13A (B) mRNA levels compared to PP Biomesh P1® (Blue) vs. without mesh (Black)*

REFERENCES

1. Kokotovic D. et al. JAMA. 2016;316(15):1575-1582. 2. Brown B. N et al. Acta Biomater. 2012; 8(3): 978–987. 3. Molina C. P. et al. J Immunol Regen Med. 2019; (3) : 13-25. 4. Deng et al. J Immunol. 2012 Oct 1; 189 (7):3669-80.