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Molecular Insights Into the Effects Of PLLA-SCA on Gene Expression and Collagen Synthesis in Human 3D Skin Models Containing Macrophages

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ABSTRACT

Injectable poly-L-lactic acid (PLLA-SCA) is used for the correction of shallow to deep nasolabial fold contour deficiencies, cheek wrinkles, and other facial wrinkles. In contrast to hyaluronan (HA) fillers, PLLA-SCA has a biostimulatory effect by activating resident fibroblasts to produce collagen, but the mechanisms are not known in detail at the molecular level. Therefore, our aim was to investigate the molecular effects of PLLA-SCA in a comprehensive in vitro study. Since PLLA-SCA-dependent collagen production in fibroblasts depends on the interaction with macrophages, we generated novel macrophage-containing 3D skin models. According to the clinical application, PLLA-SCA was injected once into the dermal equivalent of the 3D skin model. Histological analysis showed a significant increase in epidermal thickness in these models after 5 and 14 days. Gene expression profiling revealed an upregulation of integrins and laminins (e.g., LAMA3, ITGA6), which are essential components of the dermal-epidermal junction. In addition, we found an upregulation of cytokines and chemokines (TGFB2, CXCL6, IL1B) at day 14 after PLLA-SCA injection. Interestingly, immunohistochemical analyses exhibited a significantly stimulated collagen I production in our models. These effects might be attributed, at least in part, to the upregulation of IL1B and subsequently CXCL6, which stimulates collagen I synthesis in human dermal fibroblasts as we could demonstrate. Taken together, our data provide for the first time molecular insights into the biostimulatory effects of PLLA-SCA on collagen I production in novel human 3D skin models comprising macrophages.

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INTRODUCTION

Poly-l-lactic acid (PLLA-SCA) is a synthetic polymer used as an injectable to restore volume and stimulate collagen formation.[1] It was initially approved by the US Food and Drug Administration (FDA) in 2004 under the name Sculptra® (Galderma) for the treatment of HIV-associated lipoatrophy.[2] Later in 2009 it was approved as Sculptra® Aesthetic for the correction of shallow to deep nasolabial folds and other facial wrinkles in immunocompetent patients.[3] In 2023, the FDA has approved Sculptra® for the correction of cheek wrinkles.[4] PLLA-SCA has a biostimulatory effect by activating resident fibroblasts to produce collagen.[3,5] Animal experiments revealed that after injection, PLLA-SCA induces a response through phagocytosis by tissue macrophages and then slowly converts into lactic acid monomers, which are metabolized into carbon dioxide or incorporated into glucose while stimulating the production of new collagen type-I fibers in the skin.[1,6] However, the underlying molecular mechanisms are not yet known in detail. Since our previous study aimed to better understand the molecular effects of HA-based fillers with and without subsequent additional fractional laser co-treatment,[7] we now focused on gaining molecular insights into the stimulatory effects of PLLA-SCA injections on collagen I production in novel human 3D skin models comprising macrophages.

MATERIALS AND METHODS

In this in vitro study, the PLLA-SCA filler Sculptra® was injected into previously described human full-thickness 3D skin models,[8] in which macrophages were incorporated. Macrophages were isolated from peripheral blood mononuclear cells (PBMCs) by plastic adherence as published before[9] and added to the models on day 2 of culture. Sculptra® is composed of 150 mg of PLLA-SCA microparticles with a median particle size of approximately 50 µm suspended in sodium carboxymethylcellulose (NaCMC).[10] After one single injection of 100 µl Sculptra®, models were harvested after 5 and 14 days for histological and gene expression analyses. Untreated models were used as negative controls. Experiments were performed three times independently with three different cell donors.

Microarray analysis was performed as previously described[11] by using Clariom™ S assays (Thermo Fisher Scientific). Immunofluorescence staining was done using an anti-collagen I antibody (ab34710; Abcam, Waltham, MA).

For monolayer experiments, primary dermal fibroblasts were stimulated with human recombinant CXCL6 (50 ng/ml) for 24 hours.

Statistical analysis was performed using the Mann-Whitney U test. Values of $P<0.05$ were considered statistically significant.

RESULTS

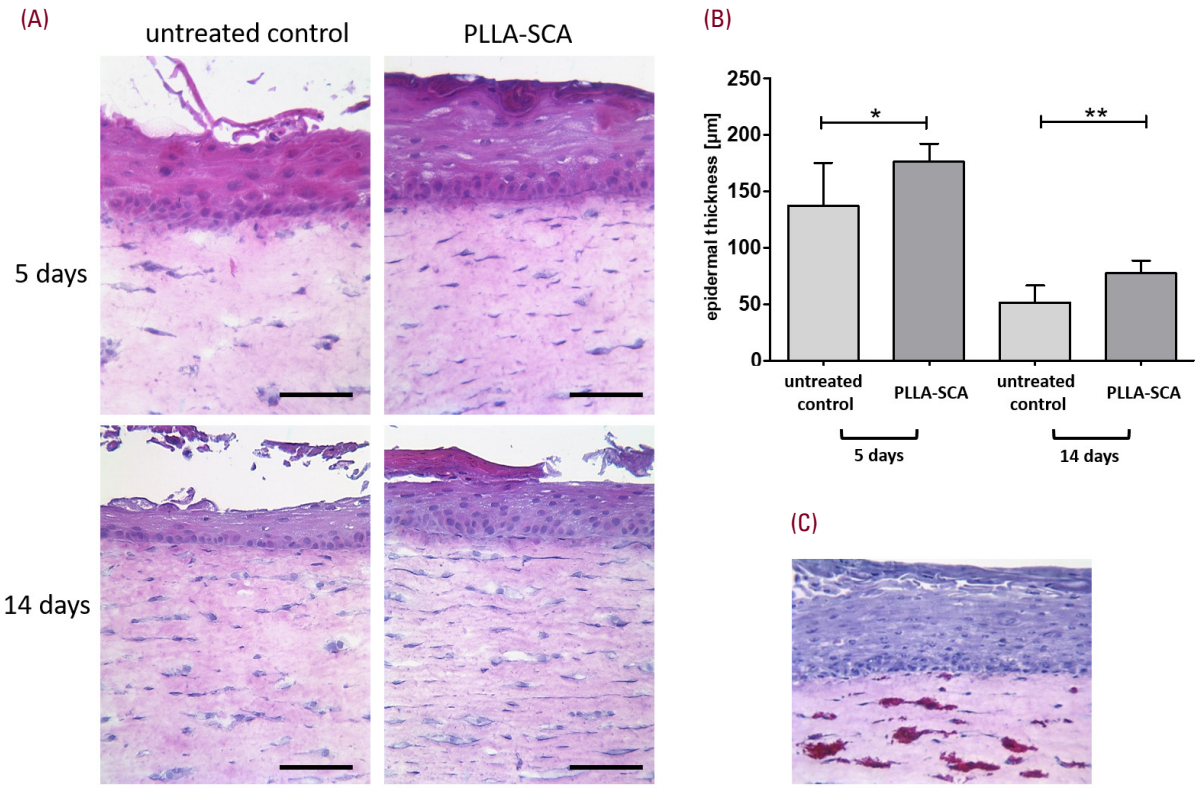
Histological analysis revealed a significantly increased epidermal thickness in our macrophage-containing 3D skin models at days 5 and 14 after PLLA-SCA injection compared to untreated controls (Figure 1A and B). Using immunohistochemical staining, we could prove that our models contained CD163-positive macrophages (Figure 1C).

On the molecular level, microarray analyses showed an upregulation of integrins (ITGA6), laminins (LAMA3, LAMC2), and desmogleins (DSG2) at day 14 after PLLA-SCA injection into the models, compared to untreated controls (Figure 2). Furthermore, we found an upregulation of cytokines (TGFB2, IL1B) and chemokines (CXCL6).

Focusing on the biostimulatory effects of PLLA-SCA on collagen production, we performed an immunofluorescence analysis of collagen I expression (Figure 3A). Quantitative fluorescence measurements revealed a significant upregulation of collagen I at day 14 after PLLA-SCA injection (Figure 3B).

To test whether CXCL6, which was upregulated in our microarray analysis, could be a potential stimulator of collagen I in the human skin, we stimulated primary dermal fibroblast monolayers with a human recombinant CXCL6 protein. An ELISA assay revealed an upregulation of collagen I in dermal fibroblasts after CXCL6 stimulation for 24 hours (Figure 3C).

FIGURE 1. (A) Representative HE stained sections of 3D skin models on day 5 and day 14 after intradermal injection of a poly-l-lactic acid (PLLA-SCA)-based filler. (B) Measurement of epidermal equivalent thickness on days 5 and 14 after PLLA-SCA injection. (C) Representative immunohistochemistry staining of CD163-positive macrophages within the 3D skin models.



Data are given as arithmetical means ± standard deviation; *$P<0.05$, **$P<0.01$.

FIGURE 2. Representative microarray analysis shows regulation of different genes in a 3D skin model, on day 14 after PLLA-SCA injection, compared to untreated control.

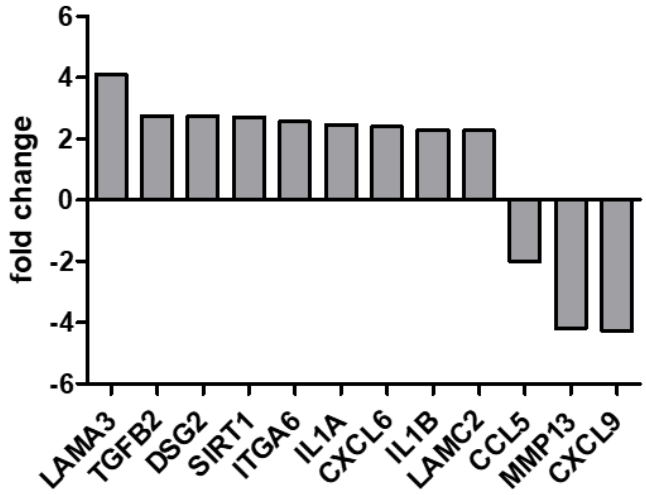
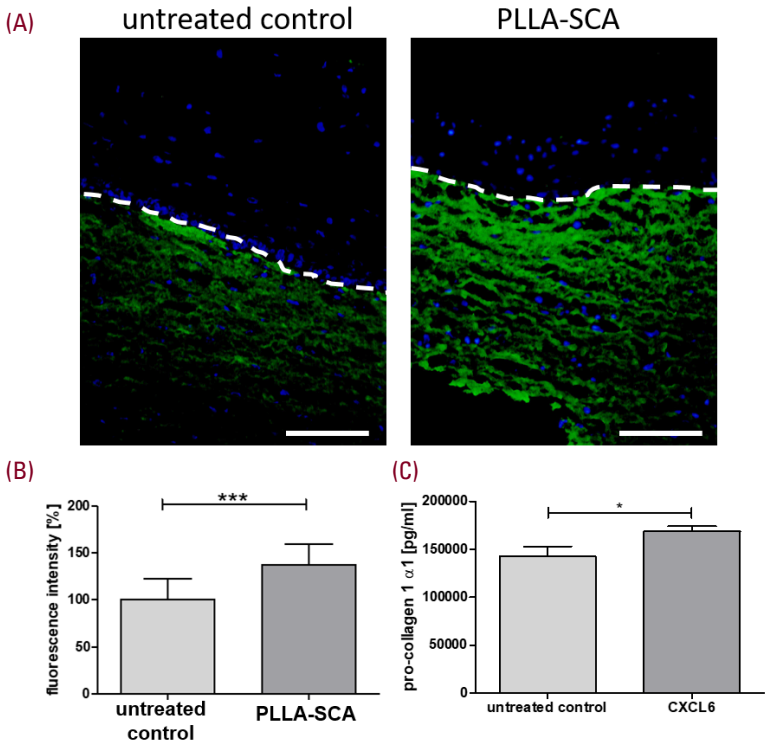


FIGURE 3. (A) Immunofluorescence examination of collagen I in 3D skin models on day 14 after PLLA-SCA injection. Untreated models served as controls. Representative images of three experiments are shown. The dashed line shows the basal membrane. (B) Quantification of fluorescence intensity, which was measured at five different representative positions per image of all experiments. ***P<0.001. (C) Pro-collagen I α1 ELISA of monolayer dermal fibroblasts that were stimulated with human recombinant CXCL6 (50 ng/ml).



Data are given as arithmetical means ± standard deviation; *P<0.05. Three independent experiments were performed.

DISCUSSION

Although several human and animal studies demonstrated the volume-enhancing effects of PLLA-SCA injections, the molecular biological effects of PLLA-SCA are only partially understood. The few in vitro studies to date on the efficacy of PLLA-SCA have used only monolayer cell cultures.[12] In our previous in vitro study, we found a stimulatory effect on epidermal thickness at day 5 after PLLA-SCA injection in full-thickness 3D skin models comprising fibroblasts and keratinocytes.[7] Gene expression profiling in these models revealed a PLLA-SCA-induced upregulation of integrins, laminins, and growth factors, among other genes.[7] Now, to investigate more deeply the biostimulatory effects of PLLA-SCA on collagen synthesis, we developed a new 3D skin model with incorporated macrophages, since it was shown that PLLA-SCA-dependent collagen production in fibroblasts occurs only in co-culture with macrophages.[12] In contrast to a previous in vitro study claiming a potentially unfavorable effect of PLLA-SCA fillers on fibroblast phenotype,[13] we did not observe any adverse effects of PLLA-SCA injection in our 3D skin models.

On days 5 and 14 after injection of PLLA-SCA into macrophage-containing skin models, we found an increased epidermal thickness at the histological level, consistent with our previous findings in 3D skin models without macrophages.[7]

On day 14 after PLLA-SCA injection, a gene expression profiling revealed an upregulation of genes expressing essential components of the dermal-epidermal junction (eg, integrins such as ITGA6, laminins such as LAMA3, and desmogleins such as DSG2). These data support the stimulatory effects of PLLA-SCA on the volume and integrity of the epidermis and especially the basement membrane. In this regard, it is interesting to note that a new study suggests a potential benefit of PLLA-SCA in the treatment of melasma where disorders of the basement membrane are involved.[14] Our data would support this potential use of PLLA-SCA in the treatment of melasma, especially by restoring basement membrane damage and upregulation of TGFb1 expression, which is known to decrease melanin synthesis via delayed extracellular signal-regulated kinase activation.[15] Further studies are needed to clarify this in detail.

Interestingly, immunohistochemical analyses exhibited a stimulatory effect of PLLA-SCA injection on collagen I production in our macrophage-containing skin models, which correlates to previous clinical findings.[16] This is the first time that these PLLA-SCA-dependent effects on collagen synthesis have been demonstrated in an in vitro 3D skin model. We assume that these effects could be attributed, at least in part, to the upregulation of IL1B and CXCL6 that we found in our gene expression analysis. In this context, previous studies indicated that CXCL6, which appears to be mainly induced by IL1B,[17] stimulates collagen synthesis in lung fibroblasts.[18] To substantiate our assumption, we have now shown for the first time that CXCL6 can also stimulate the synthesis of collagen I in dermal fibroblasts.

In summary, our data provide for the first time deeper molecular insights into the biostimulatory mode of operation of PLLA-SCA injections by performing a comprehensive in vitro study using 3D skin models containing macrophages.

DISCLOSURES

The authors have no conflicts of interest to declare.

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