Neocollagenesis after injection of a polycaprolactone based dermal filler in a rabbit

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ABSTRACT

Background: Polycaprolactone (PCL) is a well-known totally bioresorbable medical polymer that has been used in numerous CE-marked and FDA-approved medical devices for several decades. In 2010 a PCL-based dermal filler was introduced to the aesthetic market, the first dermal filler based on PCL for soft tissue augmentation providing immediate and longer-lasting results through biostimulation.

Objective: To assess the biostimulatory effects of the PCL microspheres of Ellansé™-S (Polycaprolactone; PCL-1) and Ellansé™-M (Polycaprolactone; PCL-2) through histological analysis after injection in an animal model.

Method and materials: Two rabbits were injected with different versions of the PCL-based dermal filler, PCL-1 and PCL-2. Nine and twenty-one months post-injection biopsies were taken from each injection site and sampled for histological analysis and collagen formation.

Results: Nine months post-injection the PCL-microspheres of PCL-1 were totally bioresorbed and new collagen was formed. PCL-2 revealed formation of collagen Type I and Type III around PCL-microspheres. Twenty-one months post-injection the PCL-microspheres of PCL-2 are still present in the tissue. PSR-staining confirmed the presence of primarily collagen Type I.

Conclusion: The PCL dermal filler stimulates the formation of new collagen around the smooth and spherical-shaped PCL-microspheres, leading to a mature collagen scaffold of primarily collagen Type I.

KEYWORDS: Biostimulator, neocollagenesis, collagen, polycaprolactone, injectable, dermal filler


INTRODUCTION

The use of injectable dermal fillers for soft tissue augmentation continues to be on the rise, although the attitude towards the filler type is shifting towards longer-lasting, non-permanent, biodegradable fillers¹.
Neocollagenesis is a process which has a direct influence on the longevity of dermal fillers. Biostimulating dermal fillers use the body’s natural response to encapsulate foreign bodies and to stimulate the formation of collagen around the microspheres, forming a longer-lasting implant. There are multiple types of collagen, but the most significant collagen types in the skin are Type I collagen and Type III collagen. Type III collagen is common in fast growing tissues and the first to be produced in wound-healing. In time the small collagen fibers Type III is replaced by the larger and tougher collagen fibers Type I.

In January 2010 a new biostimulating dermal filler based on polycaprolactone (PCL) microspheres (Ellansé™, AQTIS Medical, Utrecht, The Netherlands) was introduced to the aesthetic market. The Ellansé™ Family (Ellansé-S, Ellansé-M, Ellansé-L and Ellansé-E) is indicated for deep dermal and subdermal implantation.

The product is CE-marked and is made of 30% non-crosslinked PCL microspheres and 70% aqueous carboxymethylcellulose (CMC) gel carrier. It is the first dermal filler based on PCL microspheres (for review of PCL, see Gritzalas). PCL and CMC individually have an excellent and proven biocompatibility profile and have been used successfully in numerous CE-marked and FDA approved medical devices, such as dermal fillers, oral and maxillo-facial surgery, wound dressing and controlled drug delivery.

PCL is a totally bioresorbable, non-toxic medical polyester that is attractive for the use in medical devices because of its ease of bioresorption by the hydrolysis of ester linkage and the non-toxic degradation products, hydroxycaproic acid and water, which are resorbed through the metabolic pathways or readily excreted. With ³H-labeled PCL and C¹⁴-labeled PCL implantation studies it has been proven that PCL was completely excreted from the body.

The PCL microspheres are totally smooth and spherical-shaped, which has been shown to be optimal for dermal fillers. Figure 1 shows a SEM and light microscope picture of PCL microspheres.

**FIGURE 1.** SEM and light microscope picture of PCL microspheres.
picture of PCL microspheres.

After treatment the CMC gel-carrier is gradually resorbed by macrophages over a period of several weeks, during which the PCL microspheres stimulate neocollagenesis to replace the volume of the resorbed carrier. The microspheres are not phagocytosed because of their size and surface characteristics.

The PCL microspheres will trigger a natural response of the human skin and stimulate a natural wound-healing process through neocollagenesis, resulting in deposition of collagen around the microspheres. The objective of this study was to assess the biostimulatory effects of the PCL microspheres through histological and immunohistochemical analysis of skin biopsies after injection with the PCL dermal filler in an animal model.

**METHODS**

The study was performed at the Central Animal Facility of Utrecht University (GDL, Utrecht University, The Netherlands) with the approval of the animal ethical committee (DEC, Utrecht University, The Netherlands). Histological staining and analysis was performed at the Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands. The study was performed according to the ISO 10993 standard, Biological Evaluation of Medical devices, Part 6 (2007): Test for Local Tolerance Effect after Implantation.

**Dermal filler**

Two different versions of the PCL dermal filler versions were used for injection; PCL-1 and PCL-2 (AQTIS Medical, Utrecht, The Netherlands). The PCL dermal filler is composed of PCL microspheres (25-50 µm) homogenous suspended in an aqueous CMC gel-carrier. The only distinguishing characteristic of these two versions is the initial average length of the individual PCL polymer chains, which directly correlates with the time of bioresorption.

**Animals**

Two rabbits (New Zealand White) were intradermally injected lateral to the spinal column with a bolus injection of 0.1-0.2ml of the PCL dermal filler (see figure 2). Nine and twenty-one months post-injection the rabbits were euthanized by intravenous injection of sodium pentobarbital. Each of

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**FIGURE 2.** Photograph of the rabbit after injection of PCL dermal filler administered as a bolus.
the injection areas was sampled for histo-
logical analysis and preserved in 10% neu-
tral buffered formalin.

**Histological staining**

Slides were routinely Hematoxylin and
Eosin (HE) stained and picro-sirius red
(PSR) coloured. The PSR method is a selec-
tive histochemical procedure for collagen
detection in paraffin-embedded tissue and
was used as a specific staining for collagen
Type I and Type II\(^2\). This dye is known to
enhance the birefringence of collagen
fibers. The PSR bind to collagen and then
exhibit a bright refractile appearance when
transilluminated with a plane polarized light
and viewed using crossed polarizer. Non-
polarized PSR is specific for collagen,
which stains red. When polarized, large col-
lagen fibers such as Type I, color yellow to
orange birefringence, whereas smaller col-
lagen fibers such as Type III reveal green
birefringence.

**RESULTS**

**Nine months post-injection**

Figure 3 shows the photomicrograph of the
HE stained histological specimen of the in-
jected tissue 9 months post-injection of
PCL-1. The photomicrograph shows mature
dermal collagen without any traces of the
CMC gel carrier or PCL microspheres, indi-
cating that the gel carrier and the PCL mi-
crospheres are bioresorbed and cleared
from the injection site. An overview pho-
tomicrograph with non-polarized light of the
tissue 9 months post-injection of PCL-2 is
shown in figure 4. The red color staining
confirms the formation of new collagen
around the PCL microspheres resulting in a
collagen scaffold anchoring the PCL mi-
microspheres. The PCL microspheres are still homogenously distributed and no migration or encapsulation was found around the implant.

Figure 5 shows a more detailed image of the same injection tissue. Nine months post-injection, the microspheres of PCL-2 are still spherical shaped and intact at this stage of the resorption process. The photomicrograph of the non-polarized PSR-stained histological specimen confirmed collagen deposition (figure 5a). Polarized PSR staining (figure 5b) revealed the presence of both orange-red and green birefringence, documenting deposition of both Type I and Type III collagen fibers, confirming neocollagenesis.

Twenty-one months post-injection

In figure 6 an overview photomicrograph of the tissue 21 months post-injection with PCL-2 of the product is shown, with (a) non-polarized light and (b) polarized light. The microspheres of PCL-2 are still present and embedded in a collagen scaffold. Polarized PSR staining (figure 6b) shows the presence of orange-red birefringent revealing the presence of collagen Type I fibers.

The more detailed photomicrograph of the same tissue area is shown in figure 7 with (a) non-polarized light and (b) polarized light. The clear orange-red birefringent staining (figure 7b) is visible, indicating the presence of primarily large collagen fibers Type I, indicating that, as expected, over time the amount of Type III collagen declines and is replaced by the more rigid and larger Type I collagen fibers.

CONCLUSION

The data presented demonstrates the bio-
compatibility and bioresorption of the PCL dermal filler. By histological analysis it is shown that the PCL microspheres of both versions of the product induce the formation of new collagen. Due to the smoothness and spherical-shape of the PCL microparticles a scaffold of new collagen is formed.

Nine months post-injection the PCL microspheres of PCL-1 were totally bioresorbed. This is in line with the clinical study after treatment of the nasolabial folds, where the clinical efficacy seems to decrease at 12
months\textsuperscript{7,23}. The PCL microspheres of the PCL-2 are still present 9 months post-injection. The microspheres are still spherical in shape and are homogenous distributed in the tissue, without evidence of migration or encapsulation.

The PCL microspheres of the PCL-2 are still present 21 months post-injection. The PCL microspheres are embedded in a collagen scaffold of primarily collagen fibers Type I. The collagen Type III was replaced by the larger Type I collagen, in line with the normal physiological wound-healing process.

The limitation of this pilot study is the small number of animals used, and the use of a non-human model. Regardless of the small size of the study, the data shows a strong and long-term presence of collagen and the replacement of Type III by Type I, similarly observed in wound healing. More studies are needed to determine if this neocollagenesis process occurs in humans, after injection with the PCL dermal filler.

REFERENCES


