Urolon®
Brochure

urolon® *brochure*

AQLANE MEDICAL®

AQLANE Medical™ is a privately owned medical device company based in Oisterwijk, The Netherlands, focused on the provision of novel bioresorbable medical solutions that are safe and effective for the treatment of voiding dysfunctions.

Our experience and expertise lie in bioresorbable polymers and gels, and their relation to medical device safety and efficacy. Bioresorbable medical polymers are very attractive because of their ease of bioresorption by hydrolysis of ester linkages, and the non-toxic bioresorption products, which are resorbed through normal metabolic pathways and readily excreted. Such polymers have been used for several decades in various commercial FDA and CE approved medical devices. Gel components like carboxymethylcellulose are well known viscosity agents with unique elasticity and viscosity characteristics. Our core strategy is leveraging Urolon®, a novel bioresorbable urethral filler as preferred treatment option for mild to moderate stress urinary incontinence (SUI).

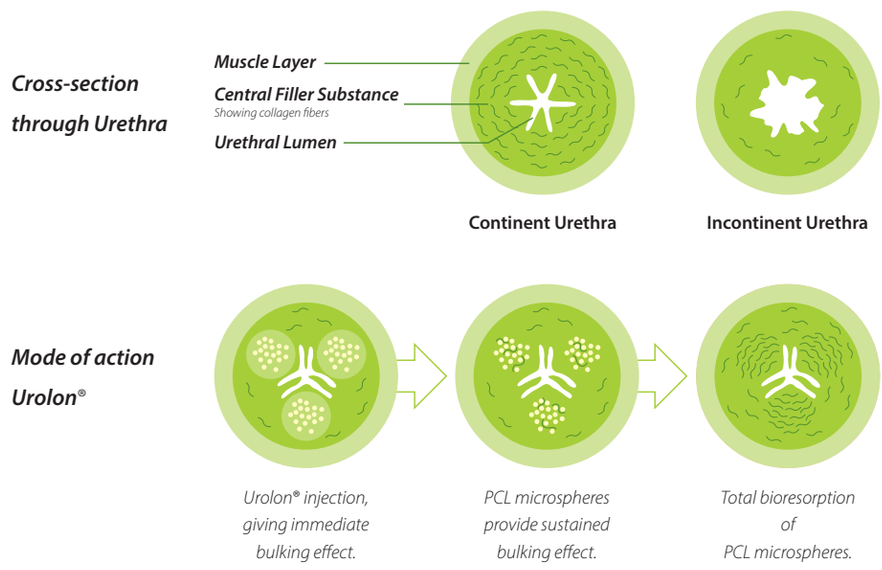
BIORESORBABLE URETHRAL FILLER; UROLON®

Urolon®, a minimally invasive treatment, is injected transurethrally into the submucosa of the urethra in order to increase urethral resistance. Data from clinical investigations have shown Urolon® is a safe and effective treatment for women suffering with mild to moderate SUI[1,1a]. Urolon® has collagen stimulation (neocollagenesis) properties[2,3]. Of note, the main constituent of the sub-urethral wall is fibrous connective tissue. Collagen of Types I, III, and VI are the predominant components of this connective tissue[4]. Women with SUI have an altered connective tissue metabolism[5-7], resulting in a decreased collagen content. Through a controlled foreign body tissue response after injection, it is suggested that Urolon® has the ability to stimulate the production of the body's own collagen (predominantly Type I) through the process of neocollagenesis, replacing lost peri-urethral collagen in SUI patients and restoring urinary sphincter function by increasing coaptation of the urethra and improving outlet resistance.

Urolon® is a non-pyrogenic, totally bioresorbable and non-permanent filler, whose principle component is synthetic polycaprolactone (PCL) microspheres suspended in a gel carrier of phosphate buffered saline (PBS), glycerin and carboxymethylcellulose (CMC). PCL is a well-known totally bioresorbable soft medical polymer and has been used in numerous CE marked and US FDA approved bioresorbable product applications for several decades and has demonstrated an excellent safety profile. Urolon® is bioresorbed through hydrolysis. This results in non-toxic bioresorption products which are resorbed through normal metabolic pathways, and fully excreted from the body[8-11]. These unique characteristics and the composition of Urolon® make it an ideal candidate for minimally invasive treatment of SUI.

Figure 1 shows the mode of action for Urolon®; upon injection the CMC gel carrier, with homogeneously dispersed PCL microspheres, gives an immediate bulking effect. After injection, the gel carrier is gradually bioresorbed, while PCL microspheres trigger a controlled foreign body response. The PCL microspheres provide the necessary volume to sustain urethral resistance during periods of increased intra-abdominal pressure. In time the PCL microspheres are totally bioresorbed and excreted from the body. It is this total bioresorption of the PCL microspheres that makes Urolon® an attractive treatment option for SUI.

Figure 1: Urolon® Mode of Action



BIOCOMPATIBILITY

According to Laeschke, optimal implant biocompatibility is reached with smooth, round and regular microspheres[12]. The Urolon® PCL microspheres are smooth, round and regular (*Figure 2a* and *2b*).

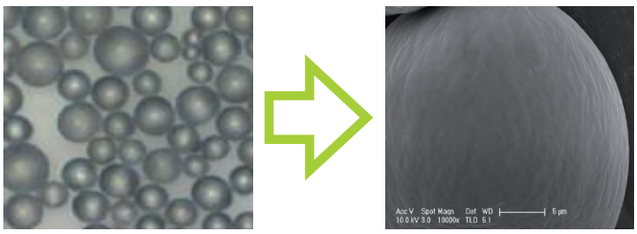


Figure 2a and 2b: Light and scanning electron micrograph (SEM) of smooth round PCL microspheres

High biocompatibility, safety and efficacy have been proven for the aesthetic equivalent of Urolon® [13,14]. Similar high biocompatibility, safety and efficacy is therefore expected for Urolon® in its use as a urethral filler. Preclinical data where PCL microspheres (in a Pluronic F127 gel carrier) were loaded with growth factors and injected around the urethra of rats, shows a high biocompatibility profile of PCL microspheres in urethral tissue[15-17].

Although the biocompatibility of PCL and CMC based devices have been extensively described in the literature, additional biocompatibility tests have been performed by NAMSA, a GLP laboratory, the Medical Animals Laboratory and Faculty of Veterinary Medicine in Utrecht, The Netherlands[18].

For the evaluation of the biological safety of the Urolon® device according to the harmonized standard ISO10993-1, the following classifications are applicable:

- Degradable implant device
- In contact with tissue/bone/blood
- Contact duration of more than 30 days is considered
- The resorption time depends on the desired efficacy of the device

Regarding the intended purpose and characteristics of the Urolon® device, according to ISO10993-1, *Table 1* shows the biocompatibility of Urolon® with regard to biological safety requirements[18].

Table 1: Biocompatibility of Urolon®

ISO10993 (GLP) Requirements	**Results/Comments**
Cytotoxicity	Non cytotoxic. *In vitro* test - article using extracts.
Sensitization	No type I sensitization in Guinea Pigs (No significant sensitization type IV).
Irritation/intracutaneous reactivity	Intra-dermal injections in rabbits. No significant irritation (similar to other dermal fillers).
Acute systemic toxicity	Intra-peritoneal injections in mice. No evidence of significant systemic toxicity or mortality.
Subacute/subchronic toxicity	Intra-peritoneal injections in rats. Local foreign body type reaction, due to slowly degrading device. No evidence of general systemic toxicity.
Genotoxicity	Non mutagenic (Ames / Bacterial Reverse mutation assay) *in vitro*.
Local tolerance and intracutaneous irritation (short-term)	Intracutaneous injection in rabbits (2wk). No significant irritation (similar to other dermal fillers), normal signs of foreign body tissue reaction due to device degradation. No evidence for migratory activity of the material at the injection site as well as in the organs investigated. The results of the clinical chemistry and hematology analyses do not indicate any effects triggered by possible migratory activity of the product.
Local tolerance (long-term)	Intradermal injection in rabbits (7 and 21 months). Normal local foreign body reaction in accordance with literature of degradable materials. Local deposits of collagen type I and III. No evidence for migratory activity of the material at the injection site as well as in the organs investigated. The results of the clinical chemistry and hematology analyses do not indicate any effects triggered by possible migratory activity of the product.
Chronic toxicity	Intradermal injection in rabbits. No systemic pathologic lesions, no additional evidence of organ toxicity, no evidence of general toxicity.
Biodegradation	Degradation behaviour, degradation products and interactions and degradation kinetics do not induce potential risks for adverse reactions.
Pyrogenicity	Intravenous injection in rabbits. Non pyrogenic.

STRESS URINARY INCONTINENCE (SUI) AND UROLON®

As mentioned earlier the main constituent of the sub-urethral wall is fibrous connective tissue. Collagen of Types I, III, and VI are the predominant component of this connective tissue[4]. As women with SUI have an altered connective tissue metabolism[5-7] (often resulting in a decreased collagen content), Urolon® has the potential to replace lost peri-urethral collagen in women with SUI, restoring urinary sphincter function by increasing outlet resistance. Furthermore, the biostimulatory properties of Urolon® in urethral tissue, are of relevance in relation to the limited longevity of other non-permanent bulking agents due to their rapid biodegradation. Additionally, the bioresorbable properties of Urolon® are paramount in relation to safety.

The quality of newly formed collagen and the biocompatibility of a particle-based stimulator depends on several characteristics shown in *Table 2*[12,19,20,21]. Urolon® was specifically designed to meet these optimal characteristics. The capacity to stimulate neocollagenesis has been proven for the aesthetic equivalent of Urolon® in both animal and human tissue[2,3].

Table 2: Characteristics that influence biocompatibility and quality of new collagen by a particle based injectable
• Size of the microspheres
• Shape of the microspheres
• Surface smoothness/roughness
• Particle volume
• Homogeneity of the microspheres in the gel
• Gel viscosity and elasticity
• Gel carrier resorption time
• Particle size distribution
• Bioresorption process

ANIMAL TISSUE ANALYSIS

In a study performed by Nicolau et al. the aesthetic equivalent of Urolon® was injected into rabbit soft tissue[3]. With the use of picro-sirius red (PSR), a specific staining for collagen Type I and Type III[22,23], the tissue was analyzed. Non-polarized PSR is specific for collagen, which stains red. When polarized, large collagen fibers such as Type I, show yellow to orange birefringence, whereas smaller collagen fibers such as Type III reveal green birefringence.

Nine-months post-injection

Figure 3 shows an overview photomicrograph with non-polarized light of rabbit tissue 9-months post-injection. The red color staining confirms the formation of new collagen around the PCL microspheres which resulted in a high-quality collagen scaffold fixating the PCL microspheres. The PCL microspheres are still homogenously distributed and no migration or encapsulation was found around the filler.

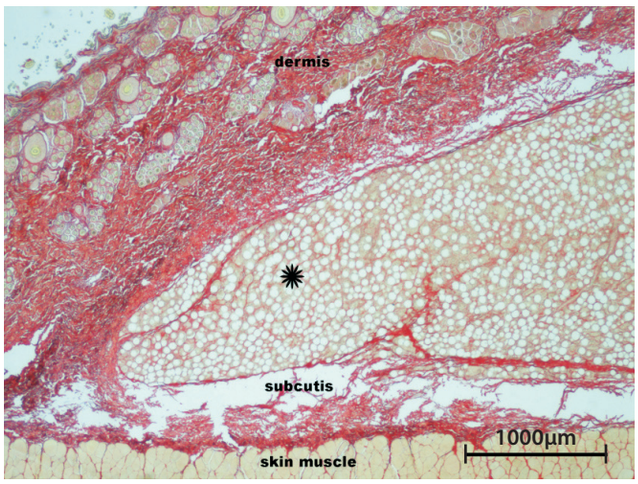


Figure 3: Photomicrograph of PSR-stained tissue 9-months post-injection with the aesthetic equivalent of Urolon® using non-polarized light. A homogenous distribution of the PCL microspheres and their fixation in the collagen scaffold is visible(*).

Figures 4a and *4b* show a more detailed image of the same injected tissue.
At 9-months post-injection microspheres 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 4a*). Polarized (*Figure 4b*) PSR staining revealed the presence of both orange-red and green birefringence, demonstrating deposition of both Type I and Type III collagen fibers, confirming neocollagenesis. The PCL microspheres are anchored in a new high-quality collagen scaffold.

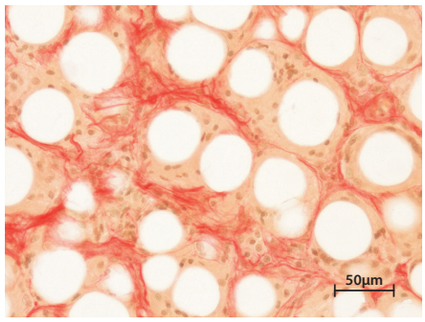


Figure 4a: Photomicrograph PSR-stained tissue 9-months post-injection with the aesthetic equivalent of Urolon® using non-polarized light. This demonstrates a newly formed collagen scaffold.

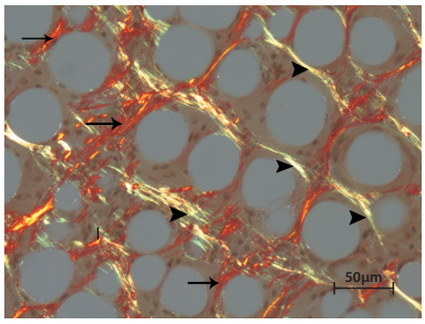


Figure 4b: Using polarized light.
The figure shows a typical orange-red and green birefringence, with red collagen Type I (arrows) and green collagen Type III (arrowheads).

Twenty one-months post-injection

Figures 5a and *5b* shows a photomicrographic overview of tissue that was injected with the aesthetic equivalent of Urolon® at 21-months post-injection, with non-polarized light (*Figure 5a*) and polarized light (*Figure 5b*). The microspheres are still present and embedded in a collagen scaffold. Polarized PSR staining shows the presence of orange-red birefringent revealing the presence of collagen Type I fibers (*Figure 5b*).

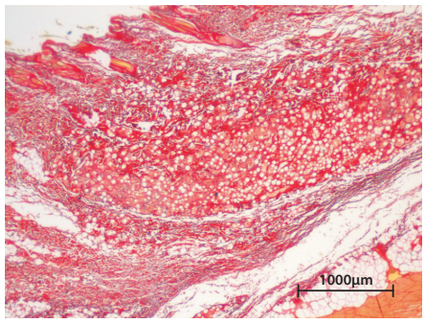


Figure 5a: Photomicrograph PSR-stained histologic specimen 21-months post-injection with the aesthetic equivalent of Urolon® using non-polarized light. The red staining confirms the formation of collagen.

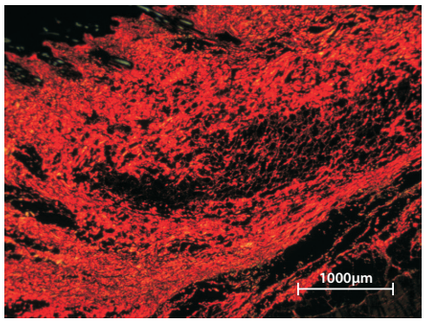


Figure 5b: Using polarized light. The red staining indicates the presence of collagen Type I fibers.

More detailed photomicrographs of the same tissue area are shown in *Figures 6a* and *6b* with non-polarized light (*Figure 6a*) and polarized light (*Figure 6b*). The clear orange-red birefringent staining (*Figure 6b*) is visible, indicating the presence of primarily large collagen fibers Type I, indicating that, as expected, during time the content of Type III collagen declines and is replaced by the more major and larger Type I collagen.

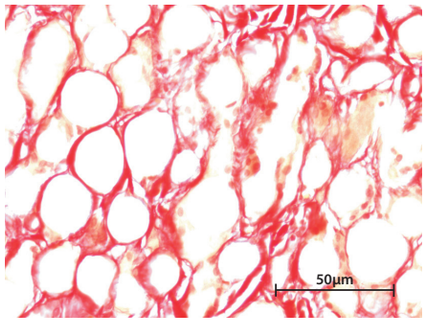


Figure 6a: Red staining confirms the presence of collagen.

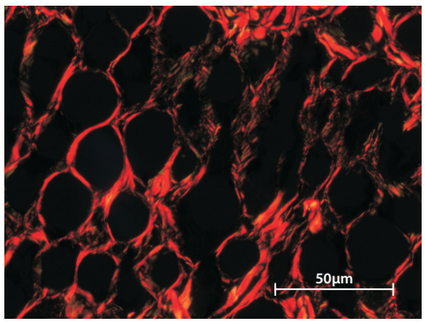


Figure 6b: Red staining indicates the presence of collagen Type I fibers.

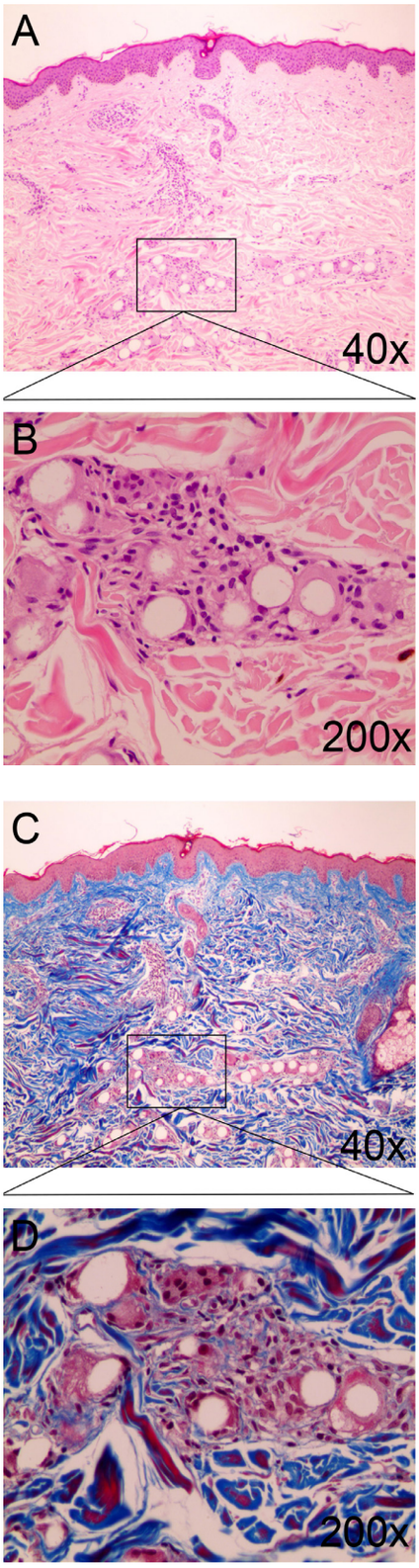


Figure 7: Microscopic images (13-months post-injection) show PCL microspheres surrounded with collagen deposition and a mild fibroblastic and histiocytic tissue response. Staining's were H&E (*a* and *b*) and Masson's Trichrome (*c* and *d*).

HUMAN TISSUE ANALYSIS

Results from the above study using the aesthetic equivalent of Urolon®, showed induced neocollagenesis which resulted in a high-quality collagen scaffold fixating the PCL microspheres into the rabbit soft tissue. A similar study performed by Kim et al. provided supportive evidence of neocollagenesis induced by the aesthetic equivalent of Urolon® in human soft tissue[2].

Histological analysis with human biopsies obtained at 13-months post-injection showed that the PCL particles appearing as perfectly smooth, round, white spheres, and measuring about 30-40 microns on average were distributed mainly in the intra-dermal layer (*Figure 7*). Furthermore, Hematoxylin & Eosin (H&E) (*Figures 7a* and *7b*) and Masson's Trichrome (*Figures 7c* and *7d*) staining revealed collagen deposition around the PCL spheres, including the presence of some histiocytes.

The data presented demonstrates the biocompatibility and biostimulatory properties of the aesthetic equivalent of Urolon® in animal and human soft tissue. By histological analysis it is shown that PCL microspheres induce the formation of new collagen, and due to optimal biocompatibility of the PCL microspheres, a high-quality scaffold of new collagen is formed. PCL microspheres are still present 9- and 21-months post-injection. The microspheres are still spherical in shape and are homogenously distributed within the tissue, without evidence of migration. Furthermore, at 21-months post-injection, PCL microspheres are embedded in a collagen scaffold of primarily Type I collagen fibers. Collagen Type III were replaced by larger Type I collagen, in line with the normal physiological wound-healing process. Human tissue analysis supports the findings from the animal soft tissue study where it was found that 13-months post injection with the aesthetic equivalent of Urolon®, neocollagenesis was induced after injection into human soft tissue. While the previous studies were performed in subdermal tissue, an animal study is ongoing to confirm the biostimulatory properties of Urolon® and its ability to induce neocollagenesis in urethral submucosal tissue.

SUMMARY

Urolon® is a bioresorbable urethral filler for the treatment of SUI, which meets the recommended features required for the ideal SUI procedure[2,4]. Central to these features are the strong biocompatibility profile and optimal characteristics required for performance as a particle-based filler. The unique characteristics, bioresorption, and the composition of Urolon® make it the ideal candidate for a minimally invasive treatment for mild to moderate female SUI. The total bioresorption of Urolon® is a great advantage over permanent materials adding further value to its safety profile.

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mild SUI — **SUI Spectrum** — **severe SUI**

Urolon®

NOTES

AQLANE Medical BV
Sprendlingenstraat 50
5061 KN Oisterwijk
The Netherlands

T +31 30 760 2610
E info@aqlanemedical.com
W www.aqlanemedical.com