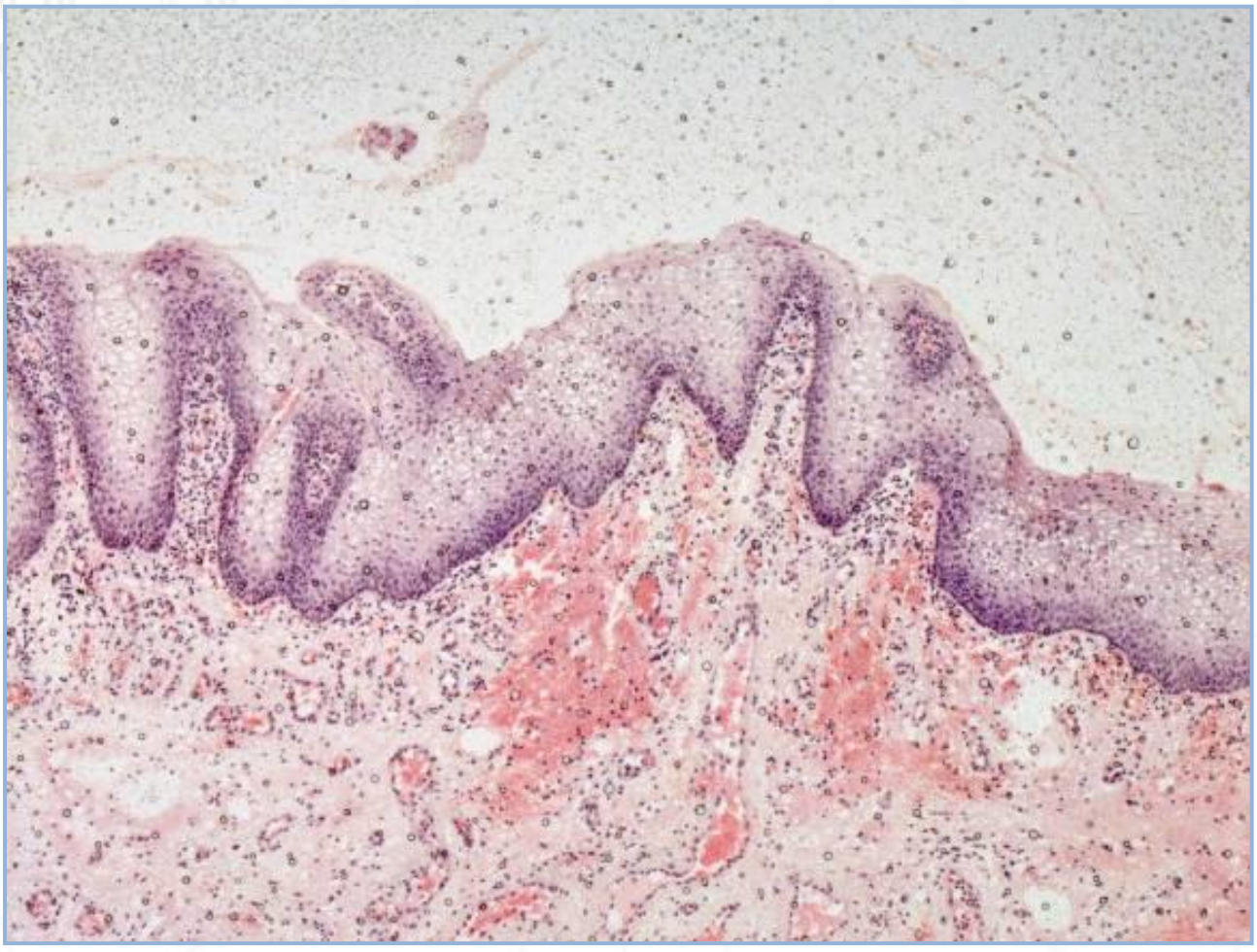
The importance of the ground matrix with respect to the vaginal mucosa.

The vagina is a canal and is the latest tract of the female genital apparatus.
It has the same structure of hollow organs and it consists of a tunica mucosa, tunica muscularis and tunica adventitia. There is no submucosa.



Histological preparation of vaginal mucosa stained with haematoxylin and eosin (H&E).
[Courtesy of Prof. A. Calligaro – University of Pavia].

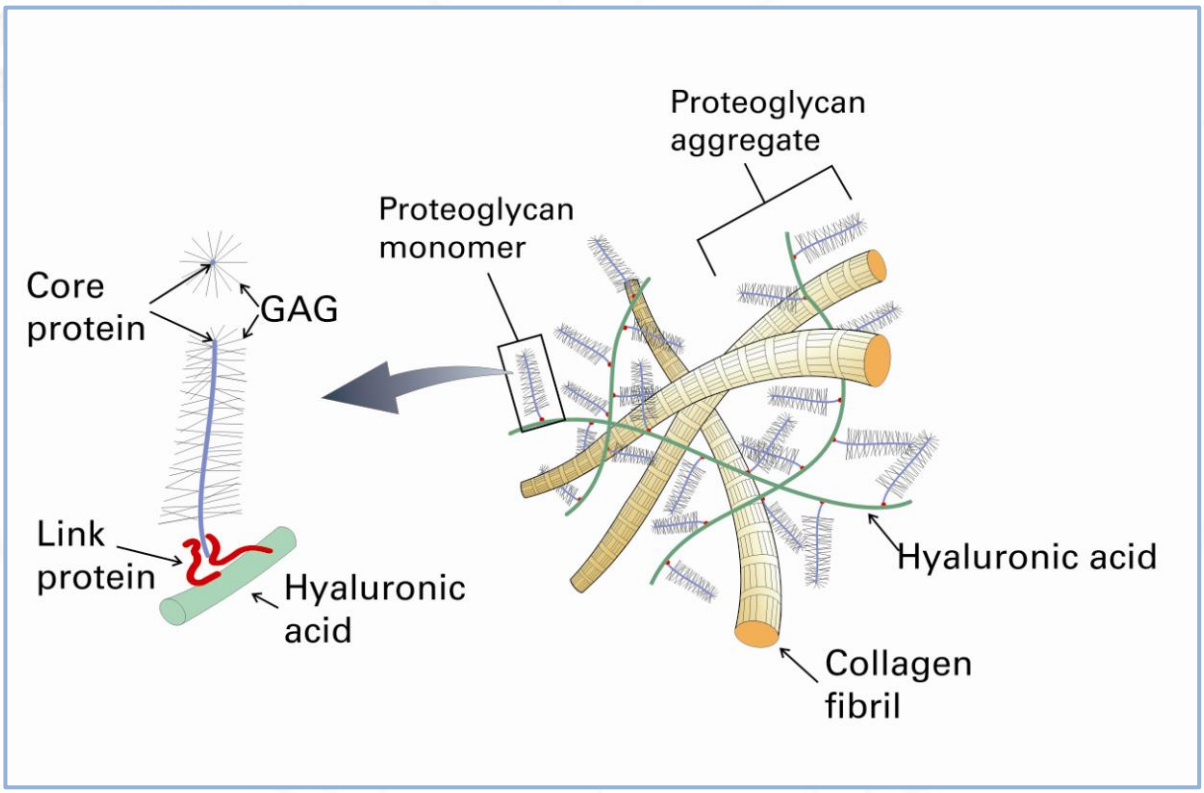
The tunica mucosa (that is to say the **vaginal mucosa**) consists of vaginal epithelium and lamina propria

- The vaginal **epithelium** is a multilayered, **non-keratinized** squamous epithelium. It regenerates thanks to a dynamic process that starts with the proliferation of the cells of the stratum basale and ends with the shedding of the most superficial cells.

- The **lamina propria** lies below the epithelium and is arranged in papillae. It consists of **connective tissue**, rich in **collagen and elastic fibres**. It contains vessels, mostly capillaries, and lymphocytes while there are no glands. It is responsible for the support and trophism (nutrition) of the vaginal lining and is fundamental for the architecture of the vaginal wall.

To better understand the mechanisms that underlie the ***MonaLisa Touch™*** treatment, it is important to consider the foundation of the connective tissue, which is usually called *Ground Matrix*.

It consists mainly of **macromolecules** known as proteoglycans, which are attached to long chains of hyaluronic acid through special proteins. Proteoglycans have the ability to **retain large quantities of water**. The high hydration level of the lamina propria depends on proteoglycans. The richness in water of the extracellular matrix means turgidity of the mucosa that cooperates with the supporting function of **well-structured collagen**. The high presence of water molecules results in a higher permeability which favours the metabolism of the mucosa in terms of an easier transport of metabolites, nutrients, etc. from capillaries to tissues (i.e. lining epithelium, connective tissue, fibres and nerve endings) and the drainage of waste substances from tissues to blood and lymph vessels. If the ground substance is poor or does not contain much water, the epithelium will not receive the nutrition necessary for its correct development or **proper hydration**.



Schematic representation of the three-dimensional structure of the extracellular matrix consisting of a fibrillar component (mainly collagen) and a ground substance.

Why does *MonaLisa Touch™ treatment work?*

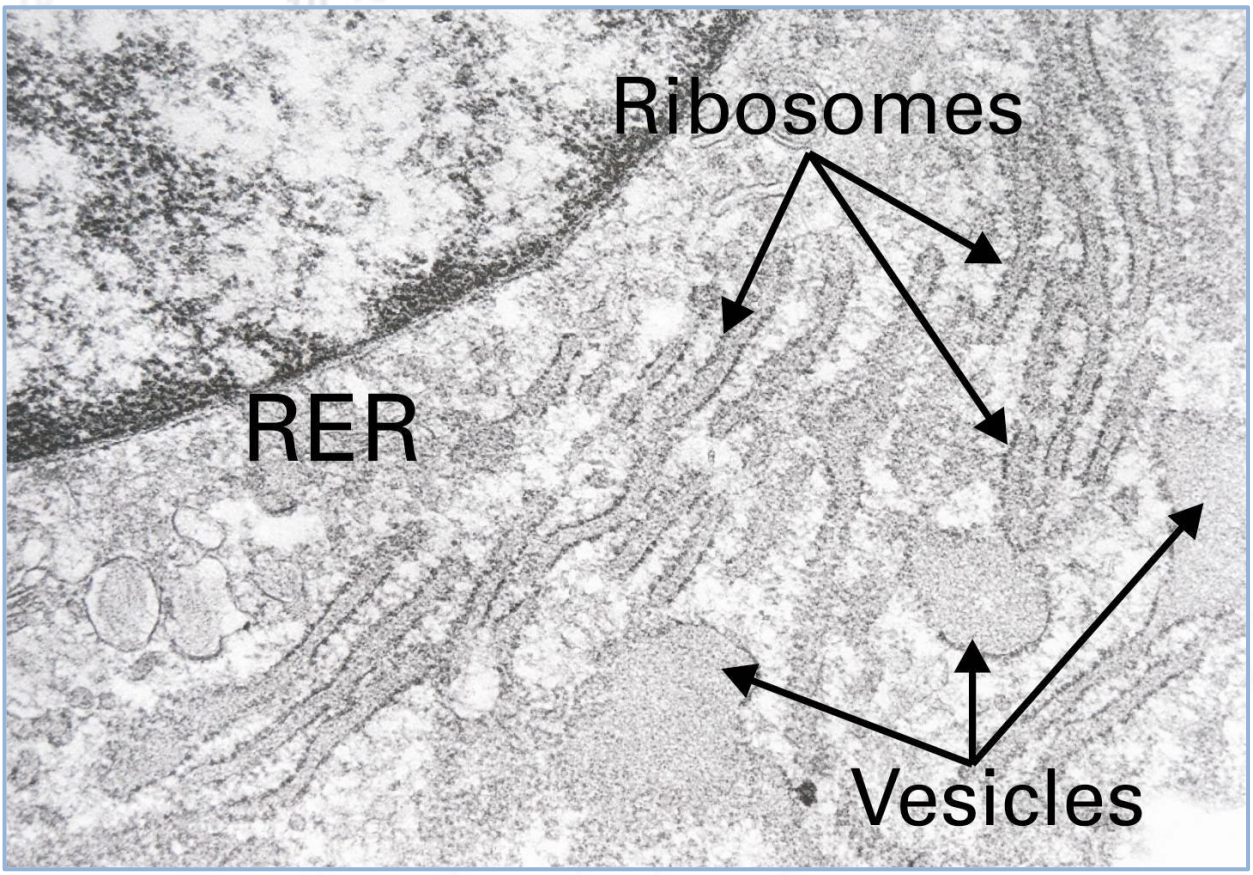
During menopause, the mucosa presents a particular condition with **fibrocytes** that are **metabolically quiescent**, as opposed to fibroblasts, and is therefore unable to actively produce hyaluronic acid and the other molecules necessary for an adequate glycoprotein ground matrix. This results in a **dry mucosa**, **which is less wet**, **less nourished** and therefore, **fragile** and more subject to **infection**. With low water content in the connective tissue, it is in fact likely for the nutrients, and the lymphocyte defenses that reach the lamina propria through the blood vessels to have greater difficulty to migrate through the connective tissue matrix to reach the epithelium, which is where infectious agents attack.

The purpose for **treating vaginal atrophy** is to promote and **recover pre-menopausal metabolic activity** by means of a new **synthesis** that does not only consist **of collagen** but also hyaluronic acid, glycosaminoglycans and proteoglycans, thereby achieving once again a hydrated and turgid mucosa with all the functionalities of **healthy and younger tissue**.

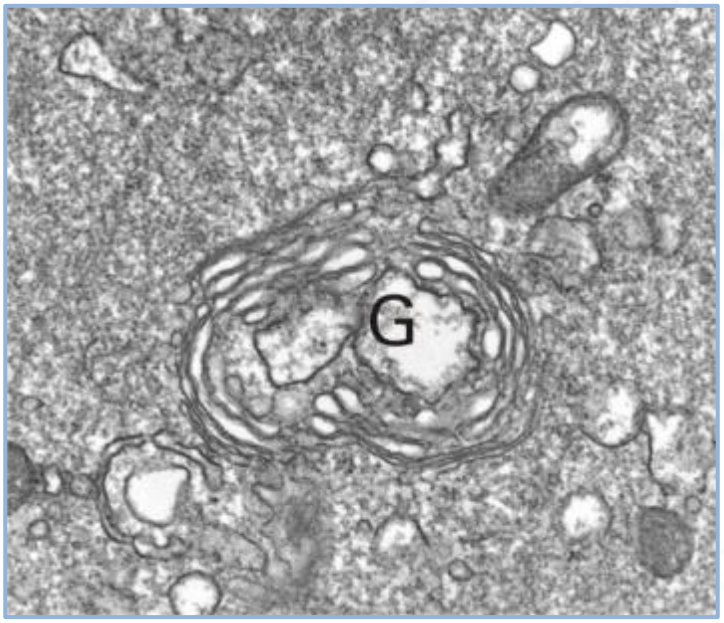
This is the stimulation effect generated by ***MonaLisa Touch™*** treatment!

In collaboration with **the San Raffaele Hospital in Milan** and **University of Pavia**, a study has been developed to look into the mechanisms triggered by ***SmartXide²*** laser treatment. The important aspect that emerged from this study lies in **the functional activation of the fibroblasts** of the vaginal mucosa, shown in the microscopic and ultrastructural observations under the electronic microscope. The biochemical mechanisms underlying these regenerative phenomena are due to the **specific thermal effect** caused by irradiation with the ***SmartXide²*** fractional CO_2 laser. Thanks to its particular emission characteristics (with the specific ***D-Pulse*** for vaginal mucosa treatment) the energy load can be transferred to the mucosa, thereby preventing excessive localised thermal damage. This is how the activation of one specific collagen chaperone ***Heath Shock Protein 47*** *(HSP47*) is induced in the fibroblasts, thereby encouraging their functional activity with renewed collagen synthesis capacity.

The morphological aspects that can be noted in the images shown on the next page are attributed to a condition with **activated fibroblasts**.



*Electron microscope image of the inside of a fibroblast in the vaginal mucosa stimulated with SmartXide² V²LR laser. The Rough Endoplasmic Reticulum (**RER**) can be seen, well-developed with many ribosomes attached to the membranes of flattened cisternae. Some of these cisternae develop vesicles in the terminal part, in which filamentous structures can be observed.*



*Electron microscope image of the inside of a fibroblast in the vaginal mucosa stimulated with SmartXide² V²LR laser. The Golgi apparatus (**G**) is also well developed. Vesicles can be observed which, we can suppose, contain the components that will form the ground matrix.*

On the basis of the innovative results attained with the preliminary study, we are planning a **new trial** on 50 patients suffering from vaginal atrophy, in order to assess the outcome of the ***MonaLisa Touch®*** treatment over a longer period of time and to quantify improvements.

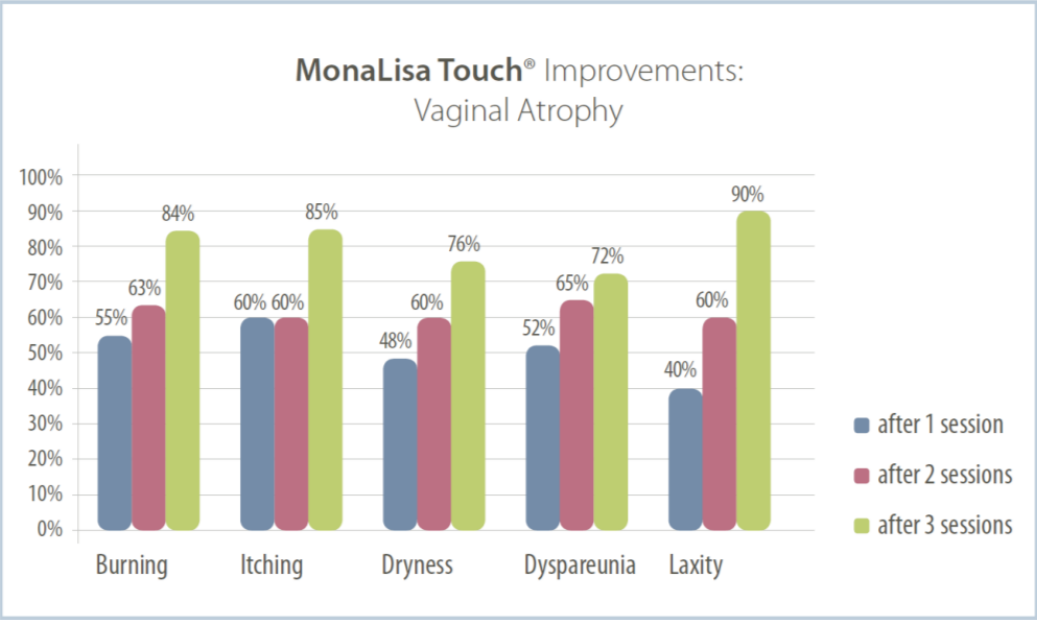
A cycle of three treatments was performed using the fractional CO2 laser system SmartXide[2].

Clinical assessments are very encouraging and show **improvements** both in terms of **symptoms** of vaginal atrophy and in general **quality of life**.

The changes in average values for single symptoms of vaginal atrophy and urinary symptoms are shown in the following tables and graphics:

	BASELINE	AFTER 1 SESSION	AFTER 2 SESSIONS	AFTER 3 SESSIONS
Vaginal Burning	5,1	2,3	1,9	0,8
Vaginal Itching	4,8	1,9	1,9	0,7
Vaginal Dryness	8,4	4,4	3,4	2
Dyspareunia	8,5	4,1	3	2,4
Vaginal Laxity	1	0,6	0,4	0,1

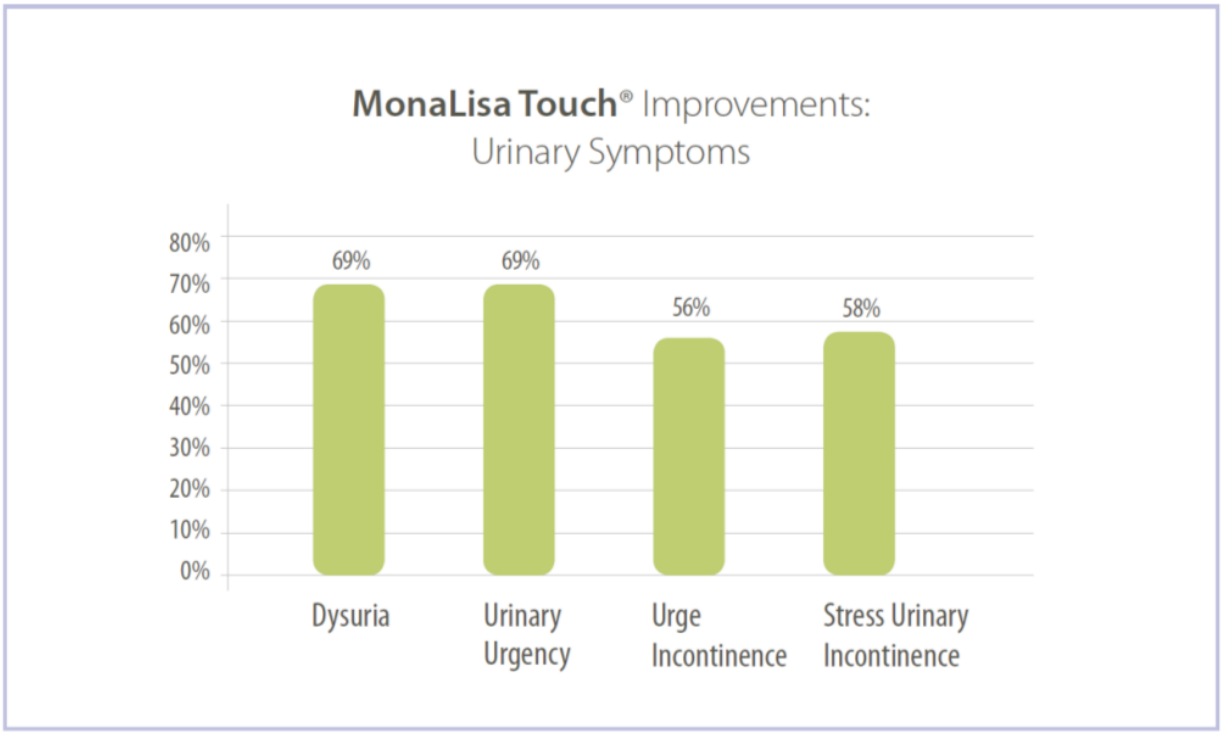
Average VAS values for main symptoms of vaginal atrophy at baseline and after each of the three individual treatments.



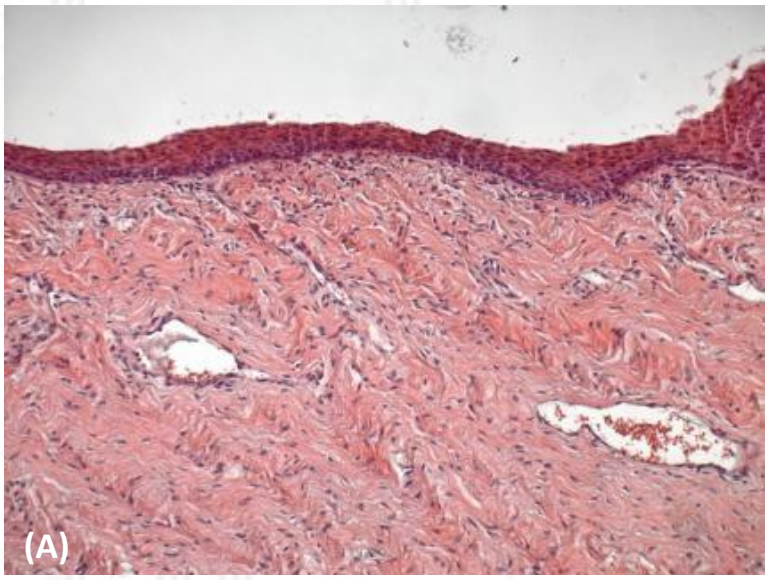
Improvement (%) for the main symptoms of vaginal atrophy after 3 MonaLisa Touch® sessions.

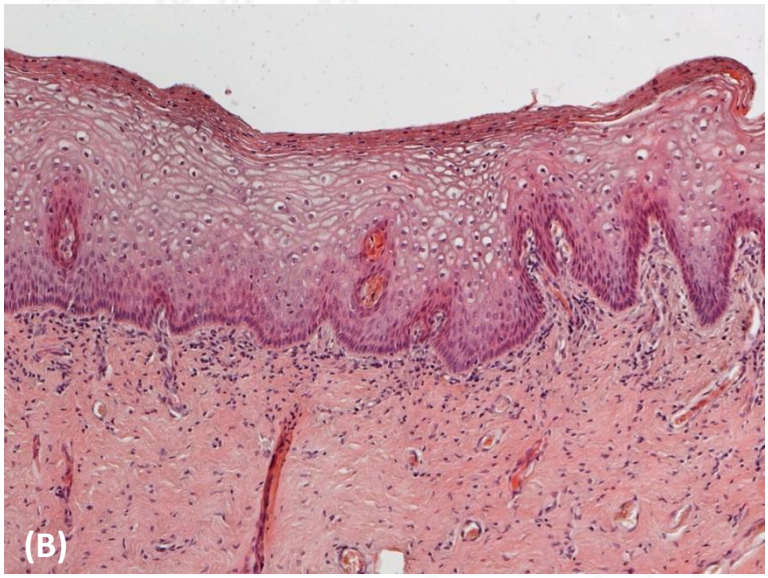
	BASELINE	AFTER 3 SESSIONS
Dysuria	1,3	0,4
Urinary Urgency	2,6	0,8
Urge Incontinence	1,6	0,7
Stress Urinary Incontinence	3,1	1,3

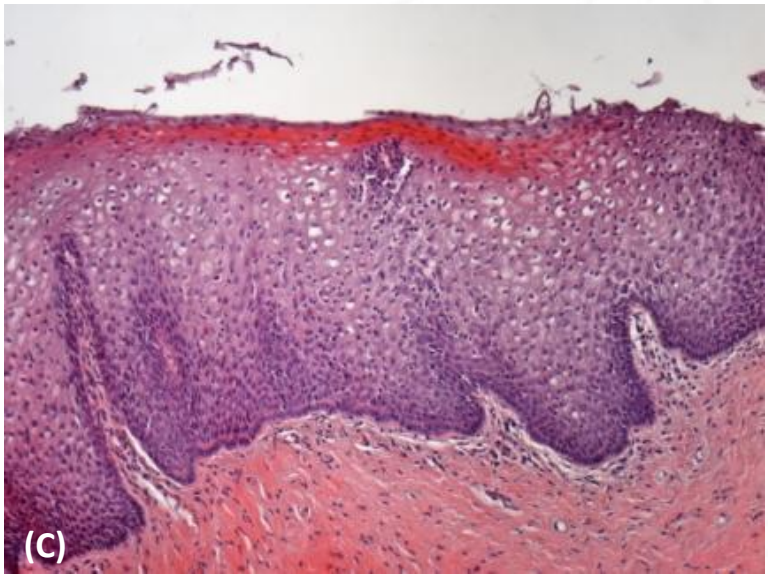
Average VAS values for main urinary symptoms, before and after 3 MonaLisa Touch® treatments.



Improvement (%) for the main symptoms of urinary incontinence after 3 MonaLisa Touch® sessions.







Histological preparation of a section of the vaginal mucosa stained with haematoxylin and eosin (H&E).
*(**A**): Vaginal mucosa in the basal condition. This morphological picture indicates vaginal atrophy at an advanced stage with an epithelium formed by few cell layers and no papillae.*
*(**B**)&(**C**): Vaginal mucosa of the same patient one month after the first (B) and the second (C) session with the MonaLisa Touch® treatment. The much thicker epithelium and the larger diameter of epithelial cells rich in glycogen, demonstrate the restored metabolic trophism and dynamics of the whole epithelium.*