

# Low Temperature Sterilization Method for Biomaterials

## MOTIVATION

There is an increasing interest in new sterilization techniques for biological materials, for instance, due to the extension of collagen scaffold usage for reconstructing various tissues such as cartilages. Preservation of the collagen scaffold structures is important with respect to its biocompatibility and stability. The latter is pre-requisite for an enhanced reconstruction of injured tissues. Thus, some standard sterilization methods using heat, steam and irradiation are unfavourable [1], [2], because they cause instability of the collagen scaffold due to thermal denaturation, thermal annealing and/or moisture treatment. Here we present a sterilization method of collagen foils based on hydrogen peroxide-gas-plasma sterilization.

## METHODS

### H<sub>2</sub>O<sub>2</sub>-GAS-PLASMA STERILIZATION

- Sterilization agent: H<sub>2</sub>O<sub>2</sub>
- Main sterilization part: Diffusion of H<sub>2</sub>O<sub>2</sub>

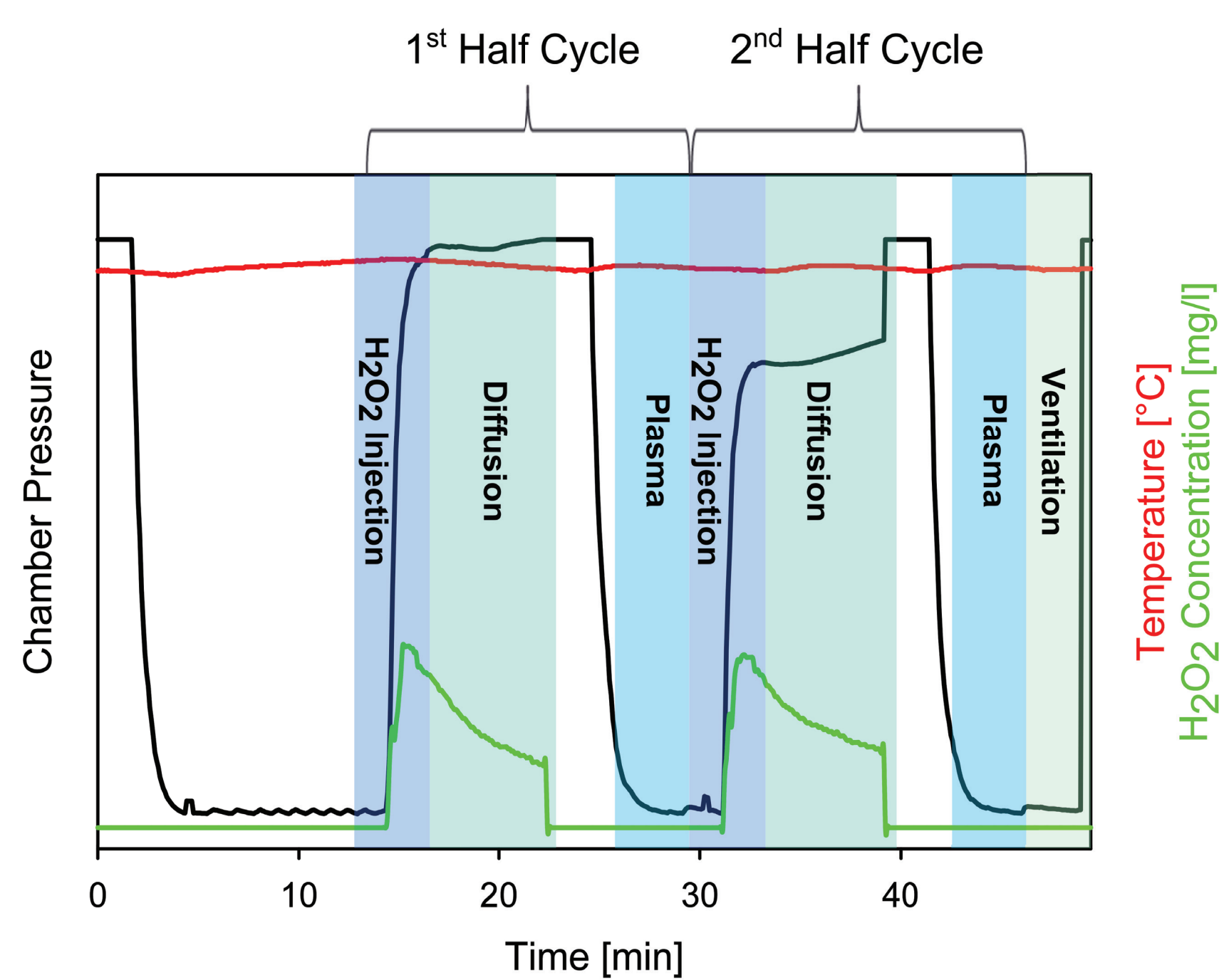


Figure 1 \ Cycles of the Gas-Plasma-Sterilization

- Ionized and compressed gas → Plasma → Reaction of H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub>

Table 1 \ Comparison between the conventional and new H<sub>2</sub>O<sub>2</sub>-plasma sterilization process

Conventional	New
<b>Chemicals</b>	<b>Chemicals</b>
• 58 w%-60 w% H <sub>2</sub> O <sub>2</sub>	• 6 w% H <sub>2</sub> O <sub>2</sub> , • 1 w% Per acetic acid (PAA)
<b>Parameter</b>	<b>Parameter</b>
• T= 50 °C-52 °C	• T= 50 °C-52 °C
• p= 62 Pa	• p= 62 Pa
• V(H <sub>2</sub> O <sub>2</sub> )=2.2 ml	• V(H <sub>2</sub> O <sub>2</sub> , PAA)=3.0 ml
• Time:	• Time:
Short cycle: 30 min	Short cycle: 30 min
Standard cycle: 45 min	Standard cycle: 45 min

### STATIC ATOMIC FORCE SPECTROSCOPY

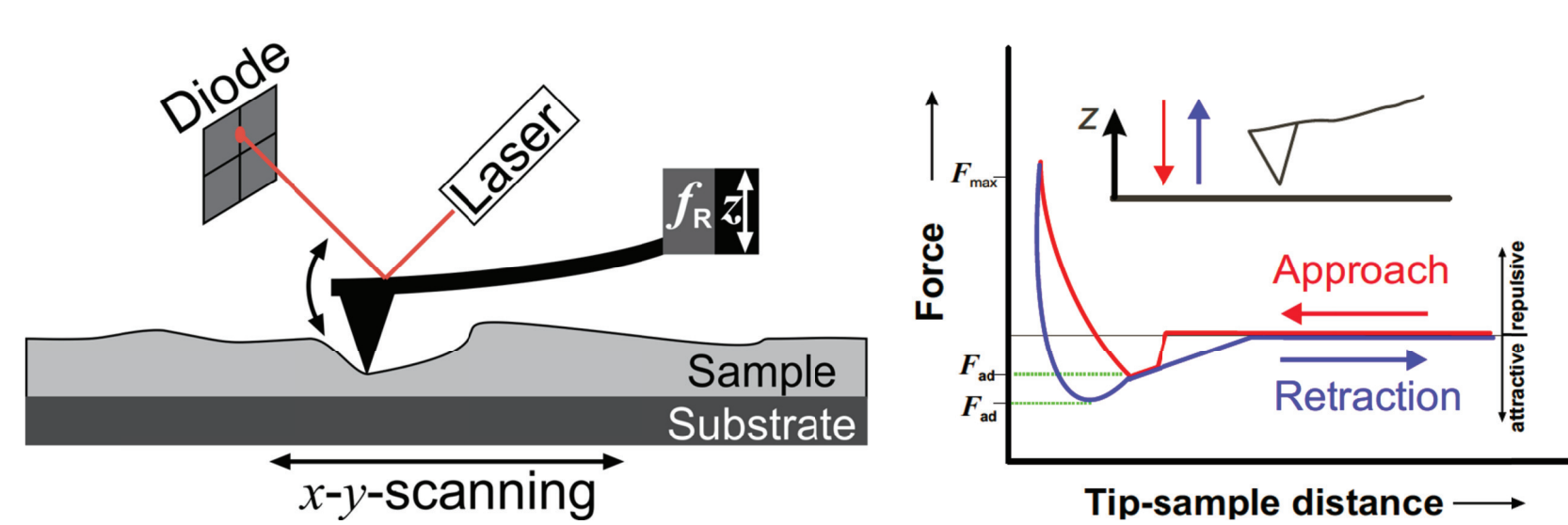


Figure 2 \ Setup and function of the atomic force microscope (AFM) and the atomic force spectroscopy

- Atomic force microscope enables a nanoscale resolution of the mechanical properties of the collagen foil.

- QI<sup>®</sup>-Mode (JPK Instruments AG) enables a simultaneous measurement of the mechanical and topographical properties in one single measurement run.

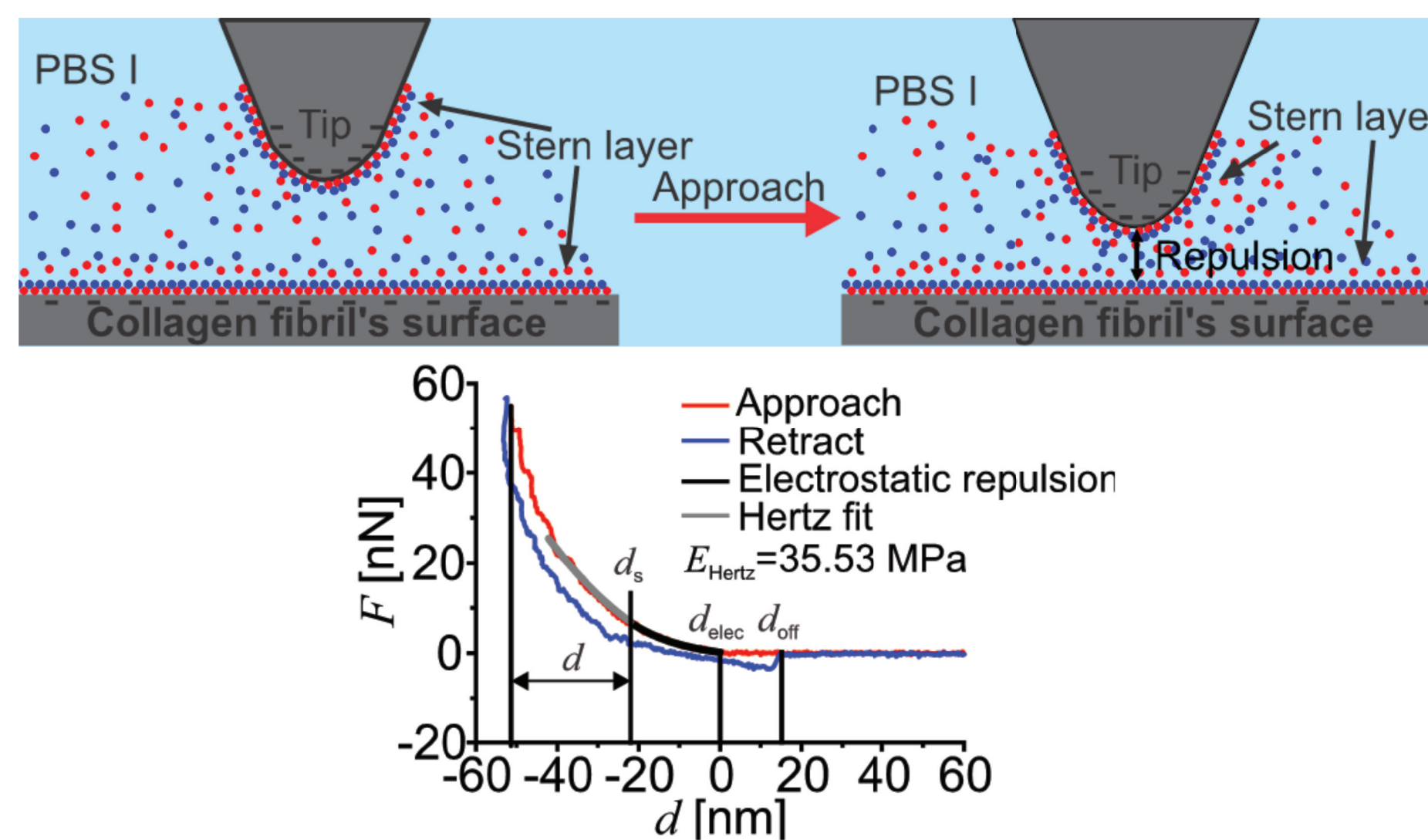


Figure 3 \ Model to reconstruct the stiffness from the force measurement on a collagen foil under physiological conditions

- Ability to measure the nano-mechanical properties under different conditions, e.g. under physiological conditions in phosphate buffered saline (PBS)

## RESULTS

### COMPARISON OF THE STANDARD- AND NEW H<sub>2</sub>O<sub>2</sub>-GAS-PLASMA STERILIZATION METHOD

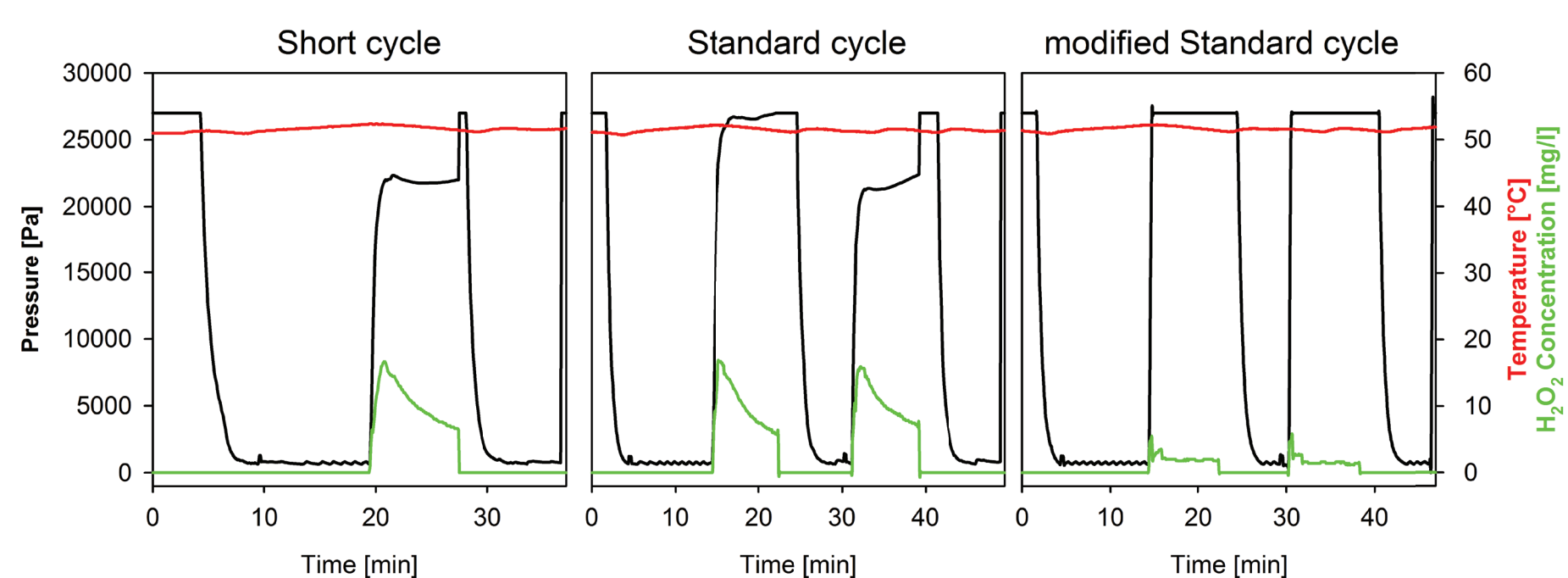


Figure 4 \ Short, standard and the new standard cycle of the H<sub>2</sub>O<sub>2</sub>-gas-plasma sterilization method

- The short cycle and the standard cycle demand a high concentration of H<sub>2</sub>O<sub>2</sub> for a successful sterilization
- The new method uses half as much H<sub>2</sub>O<sub>2</sub> as the standard and short cycle for a successful sterilization

### CHANGE OF THE NANO-MECHANICAL AND TOPOGRAPHICAL PROPERTIES AFTER DIFFERENT STERILIZATION METHODS

#### Mechanical Properties:

- Comparison of the gamma sterilization, the standard and the new plasma sterilization

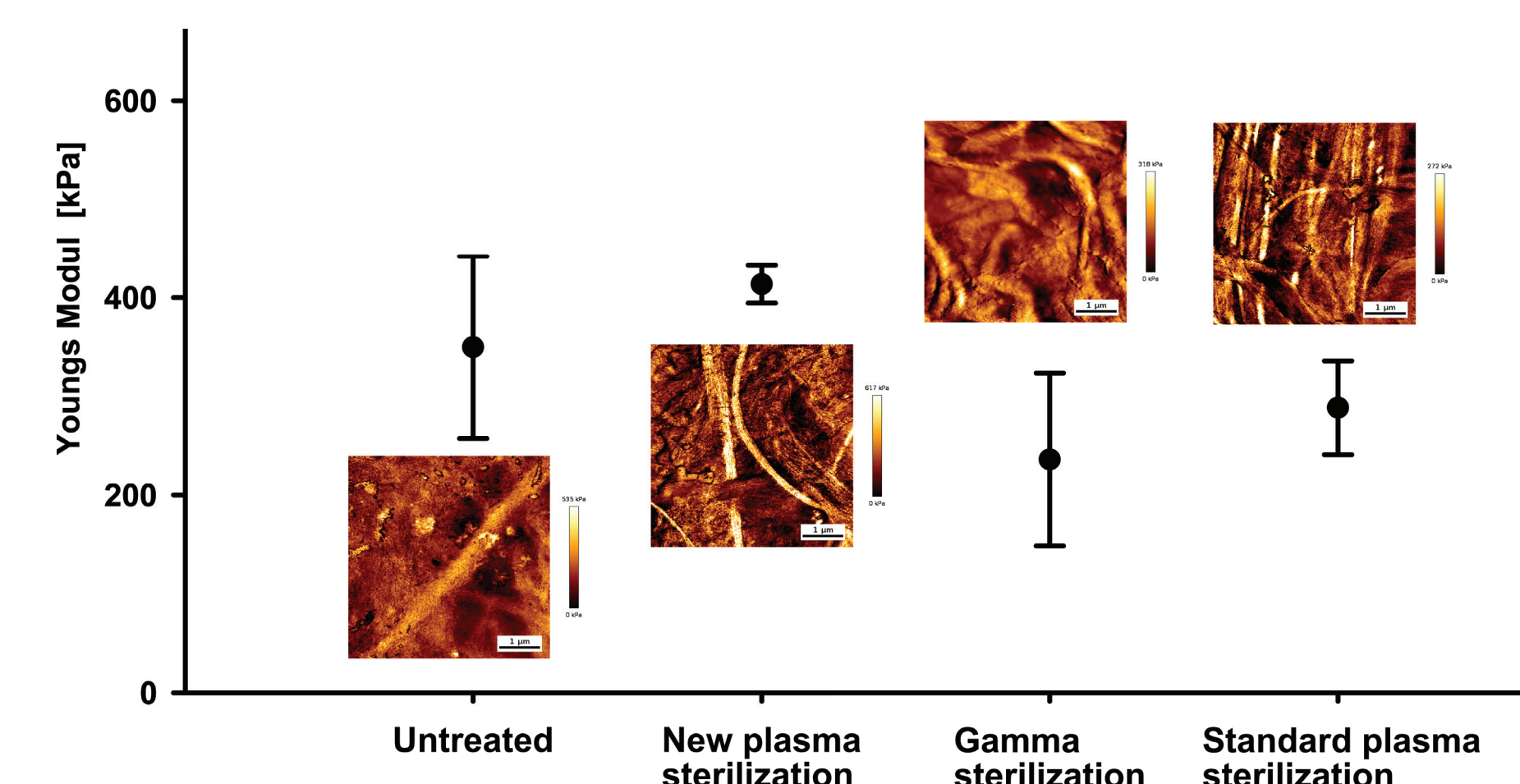


Figure 5 \ Change of the Young's module (stiffness) after gamma sterilization, standard plasma sterilization and the new plasma sterilization

- The new method does **NOT** change the mechanical properties of the collagen foil
- Gamma sterilization **does** change the mechanical properties

#### Topographical Changes:

- After gamma sterilization, the texture around the collagen fibrils appears to be smoother than untreated.

- The standard plasma sterilization and the new method do not change the morphology of the collagen fibril.
- Same texture around the fibril as in the untreated state.

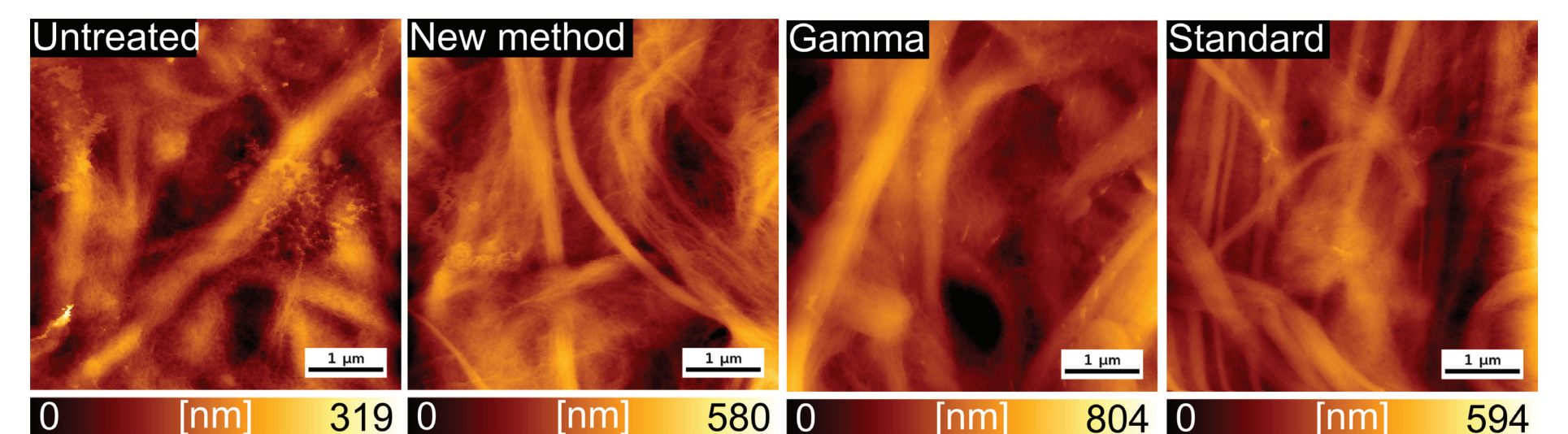


Figure 6 \ QI-mode height images from the untreated and sterilized collagen foil.

#### Physico-Chemical Analysis:

#### Differential Scanning Calometry (DSC):

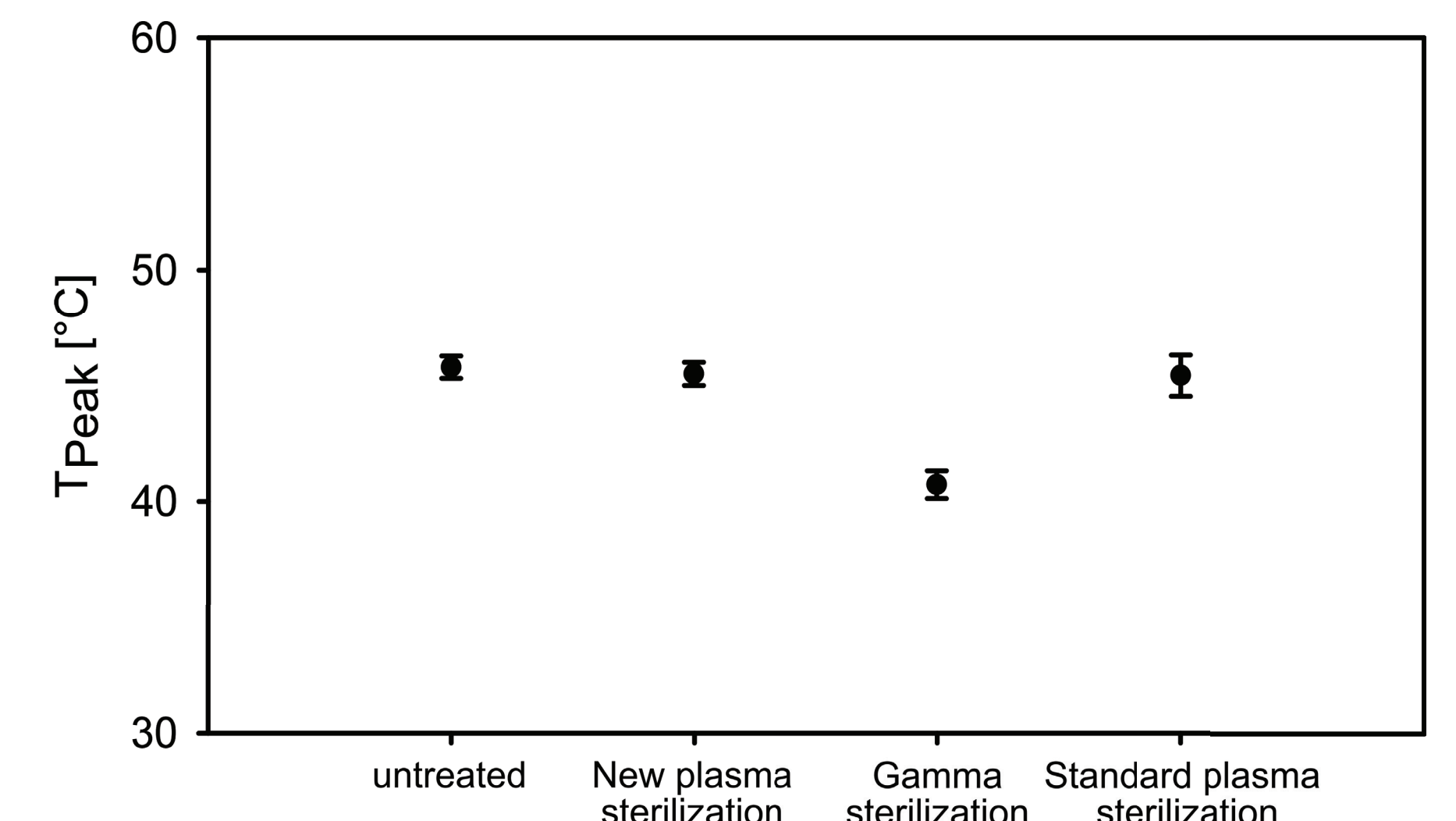


Figure 7 \ DSC measurement of the collagen foil sterilized with different methods

- No changes in the DSC are observed for Plasma Sterilization (new and old)
- Gamma sterilization denatures collagen

#### Size Exclusion Chromatography (SEC):

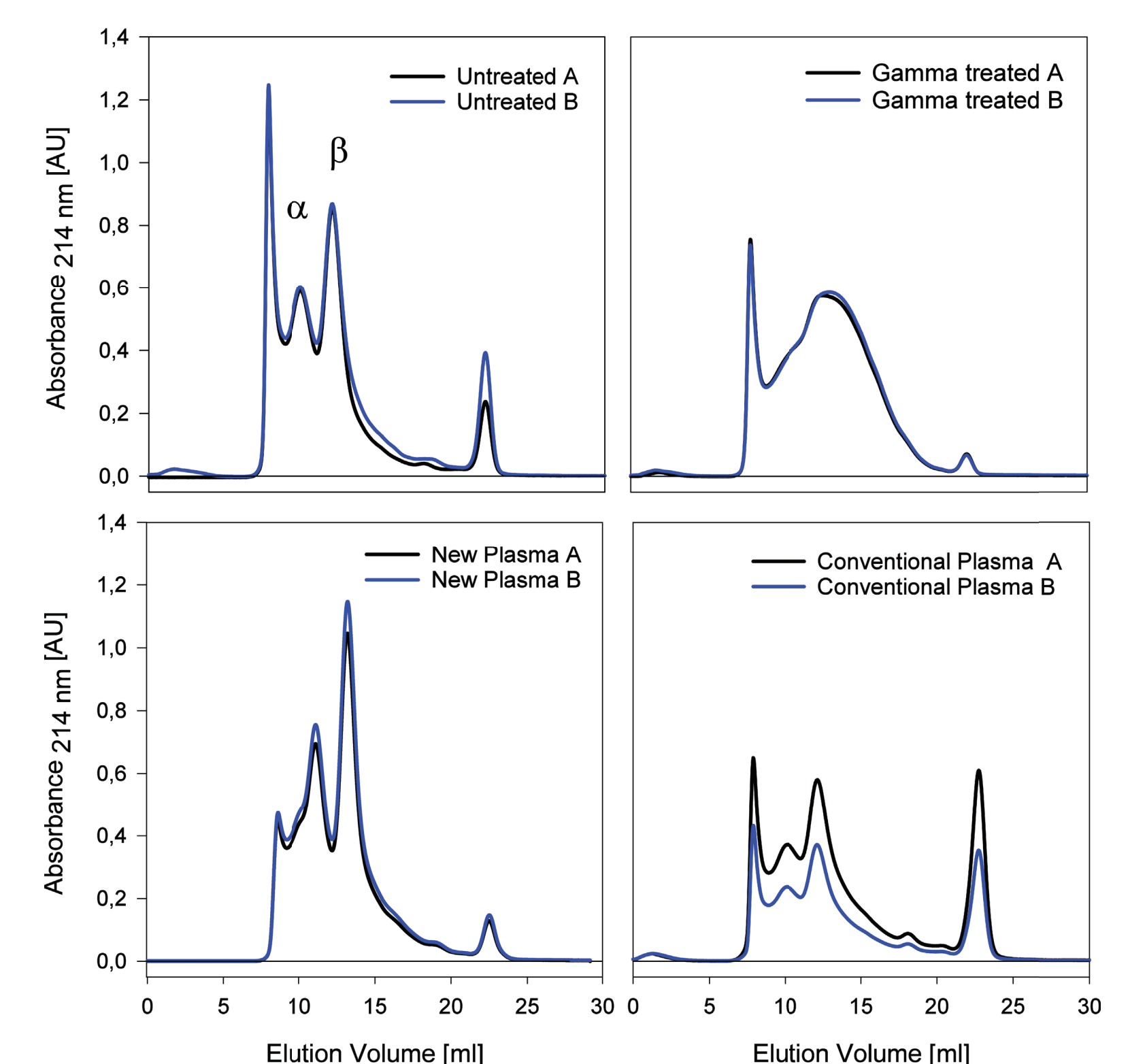


Figure 8 \ Size exclusion chromatogram of the collagen foil after different sterilization techniques

- For the new H<sub>2</sub>O<sub>2</sub>-Plasma treatment the results show, that the collagen is not denatured
- Gamma sterilization results in a cross-linked and denatured collagen foil

## CONCLUSIONS

#### The new H<sub>2</sub>O<sub>2</sub>-plasma sterilization:

- Does not alter the collagen structure
- Does not alter the mechanical properties of the collagen foil
- Is less time consuming than gamma and ethylene oxide sterilization
- Is only useable for dry materials
- Enrichment of H<sub>2</sub>O<sub>2</sub> on the surface of the collagen foil

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#### REFERENCES

- [1] E. M. Noah, J. Chen, X. Jiao, I. Heschel, and N. Pallua, 'Impact of sterilization on the porous design and cell behavior in collagen sponges prepared for tissue engineering', *Biomaterials*, vol. 23, no. 14, pp. 2855–2861, Jul. 2002.
- [2] M. J. Doherty, R. A. Mollan, and D. J. Wilson, 'Effect of ethylene oxide sterilization on human demineralized bone', *Biomaterials*, vol. 14, no. 13, pp. 994–998, Oct. 1993