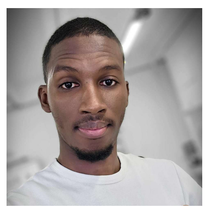
Study of the shelf-aging of PEU polymeric scaffold used in tissue engineering applications



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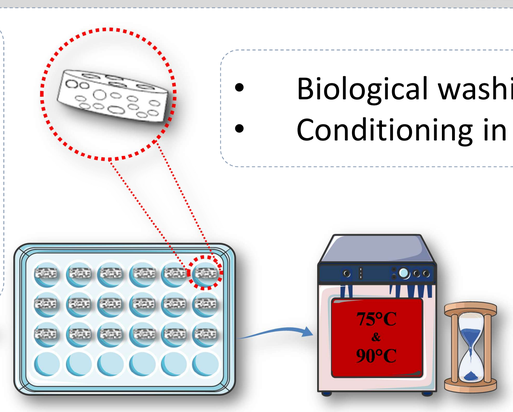
Introduction

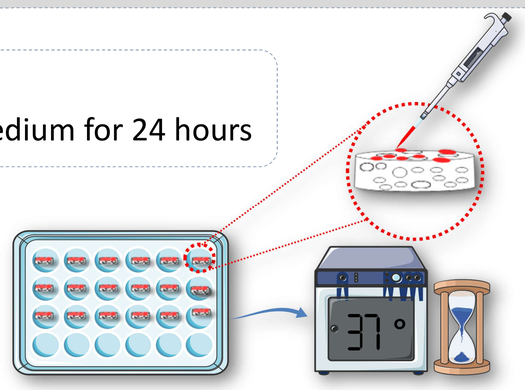
In tissue engineering applications, studies generally focus on the rate of polymeric scaffold degradation in an aqueous or cell-medium environment. Our Unit has developed a poly(ester-urethane) (PEU) elastomeric scaffold for tissue engineering applications ([1-3]) and recently observed that, after being stored at room temperature for 4 years, fibroblast cells still adhere and proliferate significantly on the aged scaffold, but stay round all over the pore surface. The aim of this study is to explore how thermally accelerated aging of PEU scaffolds impacts on the cell response.

Methods

- Synthesis of Poly(ester-urethane) scaffold using polyHIPE method
- Cutting into discs 2mm high and 1cm of diameter
- Thermal aging at 75°C and 90°C
- Chemical washing and drying

- Biological washing
- Conditioning in culture medium for 24 hours

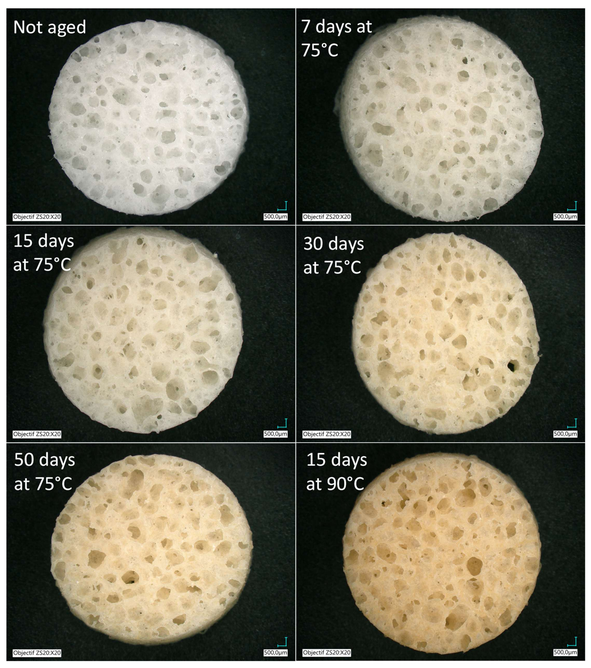


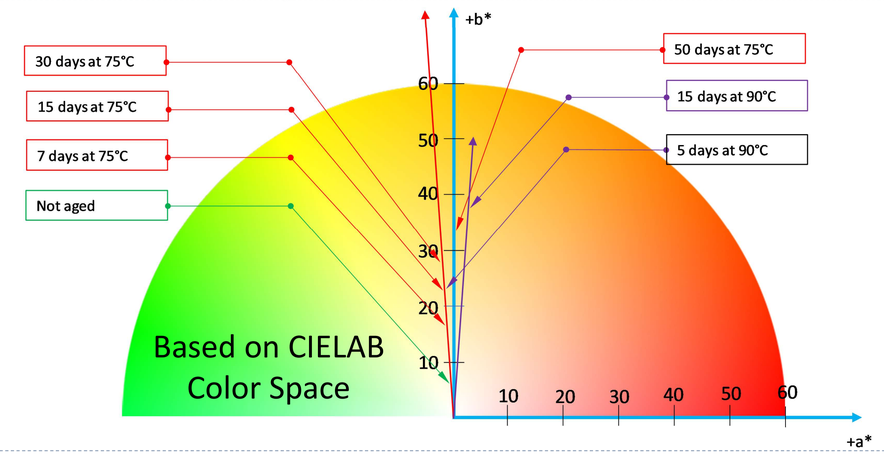


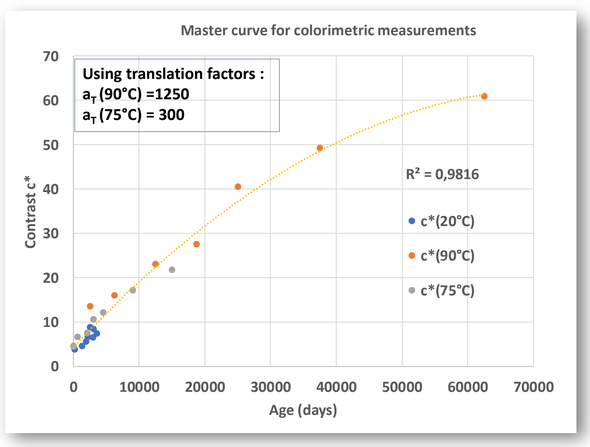
- Cytotoxicity study
- Fibroblast cell seeding and 2 hours of adhesion
- Proliferation from 7 to 40 days
- Cell counting
- Cell morphology evaluated by SEM and 3D microscopy

- Aged scaffold were characterized by : SEM/3D microscopy, colorimetry, IR/Raman/Brillouin spectroscopy

Aged scaffold characterization



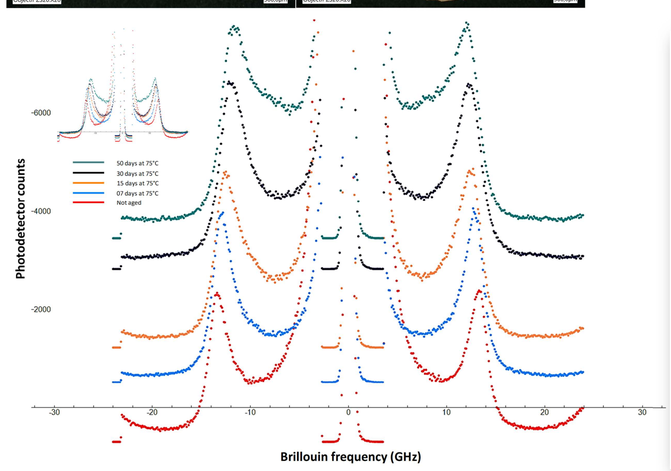


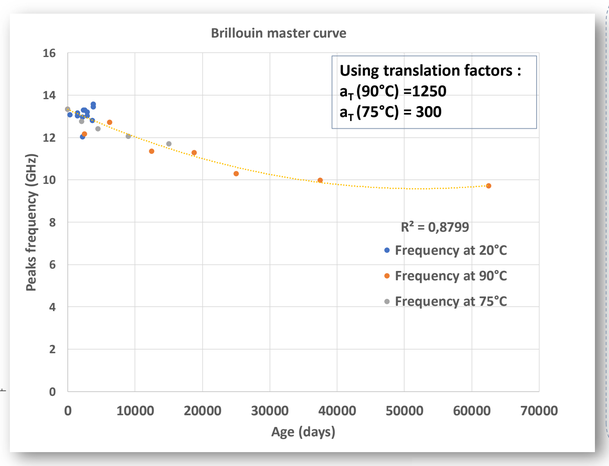


☞ Scaffolds aging caused a change in their color (color goes towards yellow).

☞ The color change is a function of the aging time, and increases very rapidly when a higher aging temperature is used (in this case 90°C).

☞ The results of the colorimetric studies (L*, a*, b* and c*) confirm this color variation.



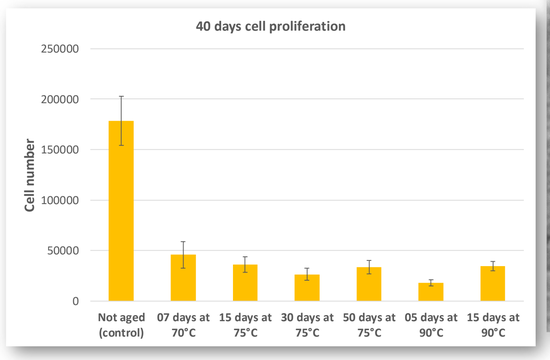


☞ FTIR analysis showed no difference, but we can consider Raman analysis to see if there is any difference. Compression tests showed no change between the scaffolds.

☞ Brillouin scattering results show that the frequency shift is both a function of aging temperature and aging time. The same observation is found for the width at half-height.

☞ These results suggest a variation in the viscoelastic properties of the scaffolds. These properties (i.e. modulus of elasticity and longitudinal viscosity) are both correlated to the full width at half-value maximum (FWHM) and peak frequency [2, 4].

In vitro tests



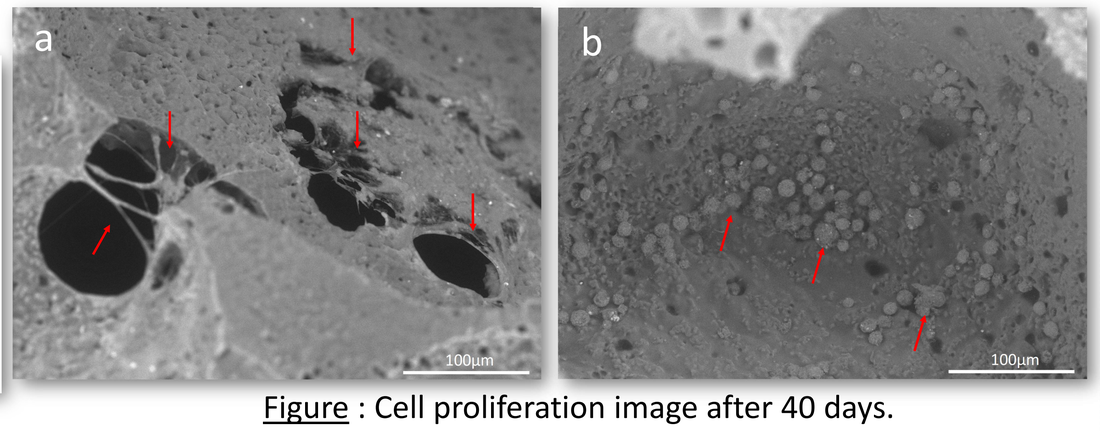


Figure : Cell proliferation image after 40 days.
a. not aged scaffold ; **b.** aged scaffold 7 days at 75°C (~ 5,7 years)

☞ Cells adhere, spread and proliferate on the non-aged scaffold as shown in **image a**.

☞ Low number of cells is explained by the fact that, after seeding, some cells fall to the bottom of the well, but they remain in good shape at the bottom of the wells.

☞ On aged scaffolds, cells adhere well but do not proliferate and remain round (**image b**)

☞ On the bottom of the wells, the cells are not in good shape (they stay round on aged scaffold)

Conclusion

The use of Brillouin scattering has made it possible to study variations in the mechanical properties of this scaffolds. The variation in these properties can be correlated with the change in color of the material. *In vitro* studies clearly show the effect of ageing on cell behavior and have already made it possible to set a storage time limit. However, aging times of 2 (~1.6 years) and 10 (~ 8 years) days at 75°C are currently being studied to confirm this limit.

Acknowledgement

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