In-vitro biodegradation of fibroin woven scaffolds for Anterior Cruciate Ligament

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Statement of Purpose: For the reconstruction of Anterior Cruciate Ligament (ACL), a biodegradable scaffold should be preferred, in order to progressively transfer mechanical loads to neoformed tissue and its degradation rate should be compatible with ligaments regeneration rate. Silk fibroin textile structures possess good mechanical properties and a macroporous structure, together with excellent biocompatibility, but only a few studies of biodegradability of complex fibroin structures are present [1]. Aim of this work was to investigate degradation kinetics of fibroin braided structures, while developing a protocol for in vitro degradation in enzymatic solution.

Methods: Braided samples were prepared with a planar pattern using 8 bundles composed of 10 silk yarns (about 80 fibroin baves) and degummed in autoclave at 120°C for 40 minutes. After 12 hours in a conditioning chamber (20°C, 60% humidity), samples were sterilized in autoclave at 120°C for 15 minutes. Enzymatic solution (ES) was prepared dissolving 1 mg of protease XIV in 1 ml of buffered solution (BS) containing 10mM Sodium Acetate and 5mM Calcium Acetate (pH 7,5) [2]. Specimens 80 mm long of braided fibroin were incubated at 37 °C in ES or BS only (respectively with 12,5 mg of fibroin/1ml for BS and 8,3 mg of fibroin/1ml for ES). Solutions were renewed every 3-4 days in order to maintain enzyme activity over 60% in the ES solution. At predetermined timepoints (up to 98 days), specimens were extracted from the solution, treated with hot water (80°C) to inactivate the enzyme (for ES samples only) and washed with distilled water at room temperature to remove residuals of salts (for both ES and BS). To assess the degradation of fibroin structures, dry weight was measured for each sample at each time point and section of fibroin baves was estimated by optical microscopy for samples extracted after 38 days (both ES and BS). Tensile tests (MTS, 1/MH) were performed with a crosshead speed of 36 mm/min on dry ES and BS specimens (gauge length 30 mm, n=4).

Results / Discussion:

Measures done on ES samples showed linear loss of dry weight (about -26 % after 98 days, Figure 1).

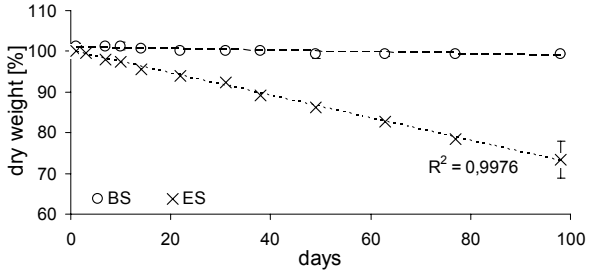

Figure 1: Dry weight of fibroin woven structures subjected to degradation

The observed weight loss was caused by both the reduction of baves section (8,46% after 38 days) and the detachment of fibroin microfibrils from single baves. For BS samples no significant differences both in dry weight and in section area was detected.

Tensile tests showed, for ES samples, a considerable reduction in maximum load and in the correspondent elongation, with a quadratic relationship (Figure 2).

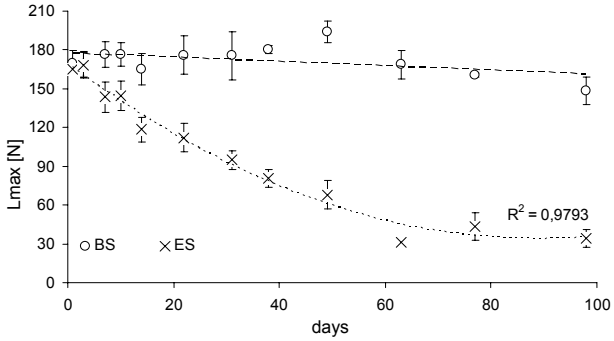

Figure 2: Maximum load of fibroin woven structures subjected to degradation

For BS samples, conversely, a significantly slower linear decrease in maximum load and elongation was found (Table 1).

	L_{max} (N)		e_{max} (mm)	
days	**BS**	**ES**	**BS**	**ES**
1	169,9±9,5	165,4±4,5	6,3±0,1	6,4±0,2
31	175,4±18,8	94,8±7,0	6,1±1,3	3,4±0,5
63	168,7±11,1	31,1±1,4	5,1±0,8	2,0±0,2
98	148,3±10,6	34,5±6,8	4,9±0,1	2,1±0,1

Table 1: Tensile properties of fibroin woven structures subjected to degradation

Conclusions:

This in-vitro study gives an insight into degradation kinetics of complex fibroin woven structures. In particular, the low decreases in maximum load and correspondent elongation, together with the negligible weight loss observed for BS samples, indicate a scant influence of hydrolysis on fibroin fibres degradation. Furthermore, correlations were found for maximum load and elongation decrease and for weight loss as a consequence of hydrolytic and enzymatic degradation.

The analysis of more complex woven, knitted or braided structures, together with *in vivo* experiments will further elucidate the degradation kinetics of fibroin fibers, and indicate the predictive validity of the *in vitro* protocol established.

References:

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[2] R.L Horan et al. (2005) Biomaterials, 25: 3385-3393