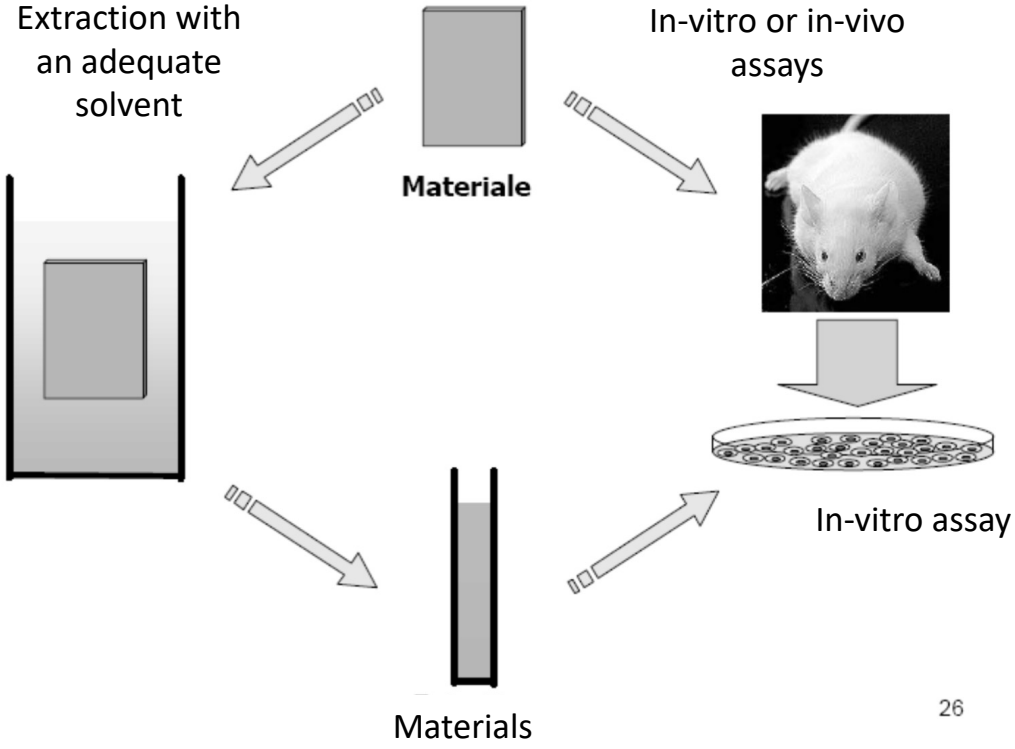
Biological Testing: Testing for evaluation

6.3.2.1 Cytotoxicity

Cytotoxicity tests employing cell culture techniques can be used to determine the cell death (e.g. cell lysis), the inhibition of cell growth, colony formation, and other effects on cells caused by medical devices, materials and/or their extracts. If testing is performed, it shall be conducted in accordance with ISO 10993-5.

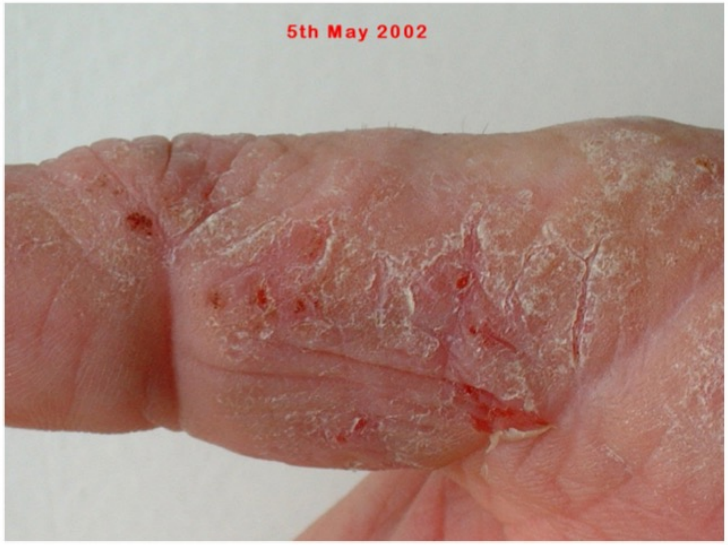


Biological Testing: Testing for evaluation

6.3.2.2 Sensitization

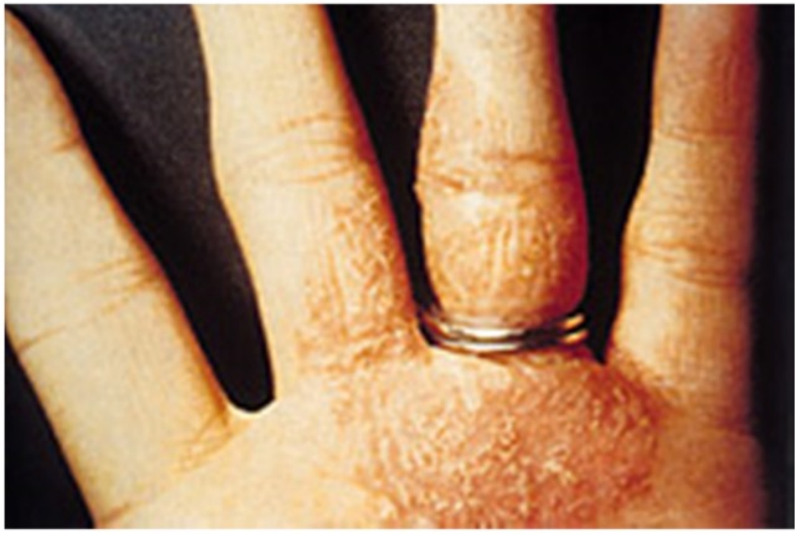
Sensitization (e.g. delayed-type hypersensitivity) tests can be used to estimate the potential for contact sensitization by medical devices, materials and/or their extracts, using an appropriate model. If testing is performed, it shall be conducted in accordance with ISO 10993-10.

These tests are important because repeat exposure or contact to even very small amounts of potential leachables can result in sensitization, which can lead to allergic reactions.



- Test di Buehler

- Test massimizzazione o di Magnuson-Kligman



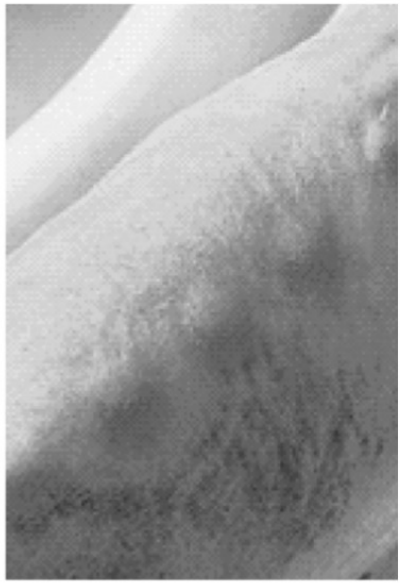
Sensitization reaction after repeated use of gloves made of of lactide

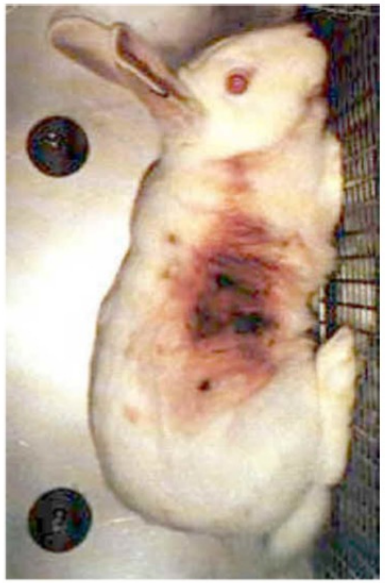
Biological Testing: Testing for evaluation

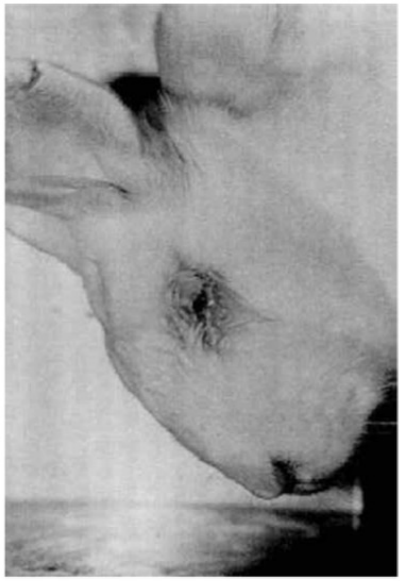
6.3.2.3 Irritation (including intracutaneous reactivity)

Irritation tests can be used to estimate the irritation potential of medical devices, materials and/or their extracts, using an appropriate site for application such as skin, eye and mucous membrane in a suitable model. The test(s) performed shall be appropriate for the route (skin, eye, mucosa) and duration of exposure or contact, and shall be conducted in accordance with ISO 10993-10.

The intracutaneous reactivity test can be used to assess the localized reaction of tissue to medical device extracts. This test is applicable where the determination of irritation by dermal or mucosal tests is inappropriate (e.g. where medical devices are implanted or have blood contact). This test might also be useful where extractables are hydrophobic (see ISO 10993-10).







Biological Testing: Testing for evaluation

6.3.2.4 Haemocompatibility

Haemocompatibility tests can be used to evaluate, using an appropriate model or system, the effects of blood-contacting medical devices or materials on blood or blood components.

One haemocompatibility test, haemolysis, determines the degree of red cell lysis and the release of haemoglobin caused by medical devices, materials, and/or their extracts in vitro.

Any testing performed shall be conducted in accordance with ISO 10993-4.

6.3.2.5 Material-mediated pyrogenicity

Pyrogenicity tests as part of a biological evaluation are intended to detect material-mediated pyrogenic reactions of extracts of medical devices or materials. No single test can differentiate pyrogenic reactions that are material-mediated from those due to endotoxin contamination (see ISO 10993-11:2017).

6.3.2.6 Acute systemic toxicity

Acute systemic toxicity tests can be used where contact allows potential absorption of toxic leachables and degradation products, to estimate the potential harmful effects of either single or multiple exposures, during a period of less than 24 h, to medical devices, materials and/or their extracts in an animal model. These tests shall be appropriate for the route of exposure, and any testing performed shall be conducted in accordance with ISO 10993-11.

Biological Testing: Testing for evaluation

6.3.2.7 Subacute and subchronic toxicity

Subacute and subchronic toxicity tests can be carried out to determine the effects of either single or multiple exposures or contact to medical devices, materials and/or their extracts for a period not less than 24 h to a period not greater than 10 % of the total life-span of the test animal (e.g. up to 13 weeks in rats).

These tests shall be waived if available data for the chronic toxicity of the relevant materials are sufficient to allow the subacute and subchronic toxicity to be evaluated. The reason for waiving of the tests shall be included in the overall biological evaluation report. These tests shall be appropriate for the route and duration of contact.

Subacute and subchronic toxicity tests, if performed, shall be conducted in accordance with ISO 10993-11.

6.3.2.8 Chronic toxicity

Chronic toxicity tests can be used to determine the effects of either single or multiple exposures to medical devices, materials and/or their extracts during a major period of the life-span of the test animal (e.g. usually 6 months in rats). These tests shall be appropriate for the route and duration of exposure or contact, and if performed, shall be conducted in accordance with ISO 10993-11.

6.3.2.9 Implantation effects

Implantation tests can be used to assess the local pathological effects on living tissue, at both the gross level and microscopic level, of a sample of a material or final product that is surgically implanted or placed in an implant site or tissue appropriate to the intended application (e.g. special dental usage tests). These tests shall be appropriate for the route and duration of contact, and if performed, shall be conducted in accordance with ISO 10993-6.

Biological Testing: Testing for evaluation

6.3.2.10 Genotoxicity

Genotoxicity tests can be used to assess the potential for gene mutations, changes in chromosome structure and number, and other DNA or gene toxicities caused by medical devices, materials and/or their extracts. A battery of in vitro tests is initially used. If testing is performed, it shall be conducted in accordance with ISO 10993-3.

6.3.2.11 Carcinogenicity

ISO 10993-3 discusses the strategy for evaluating carcinogenicity of medical devices, materials and/or their extracts over a period of the major portion of life-span of the test animal. Carcinogenicity may be addressed with a risk assessment including chemical identification of impurities, extractable or leachable chemicals, patient exposure to these chemicals, weight of evidence (WOE) and mode of action (MOA) information, if available. Carcinogenicity information should be appropriate for the route and duration of exposure or contact, and can be available from the toxicity literature. In the absence of any significant cancer risk, it is rare for carcinogenicity tests to be considered appropriate for medical devices.

6.3.2.12 Reproductive and developmental toxicity

Reproductive and developmental toxicity tests referenced in ISO 10993-3 can be used to evaluate the potential effects of medical devices, materials and/or their extracts on reproductive function, embryonic development (teratogenicity), and prenatal and early postnatal development. Reproductive toxicity evaluations shall only be conducted when the medical device has potential impact on the reproductive potential of the subject.

In addition, developmental toxicity evaluations should be considered for medical devices or their materials used during pregnancy.

Biological Testing: Testing for evaluation

6.3.2.13 Degradation

Degradation information shall be provided for any medical devices, medical device components or materials remaining within the tissue, that have the potential for degradation within the human body.

Degradation tests shall be considered if

a) the medical device is designed to be absorbable, or

b) an informed consideration of the finished medical device composition indicates that toxic degradation products might be released during body contact.

6.3.2.14 Toxicokinetic studies

The purpose of conducting toxicokinetic studies is to evaluate the absorption, distribution, metabolism and excretion (ADME) of a chemical. The need for in vivo toxicokinetic studies, to determine the processes of absorption, distribution, metabolism and elimination of leachables and degradation products of medical devices, materials and/ or their extracts (see 6.3.2.13 and ISO 10993-16), shall be considered in the light of results from the in vitro degradation studies.

6.3.2.15 Immunotoxicology

While not specifically addressed in Annex A, ISO/TS 10993-20 provides an overview of immunotoxicology with particular reference to the potential immunotoxicity of medical devices. Immunotoxicity testing shall be considered based on the chemical nature of the materials of manufacture and data from sources that are suggestive of immunotoxicological effects or if the immunogenic potential of any of the chemicals is unknown. If immunotoxicity testing is performed, it shall be conducted in accordance with ISO/TS 10993-20.

ISO 10993-13 — Part 13: Identification and quantification of degradation products from polymeric medical devices

In accordance with ISO 10993-9, degradation tests shall be used to generate, identify and/or quantify degradation products. If degradation is observed in an accelerated test, identification and quantification of the degradation products can provide sufficient information for risk analysis. If identification and quantification of degradation products from the accelerated test do not provide sufficient information for the risk analysis, real-time testing shall be performed.

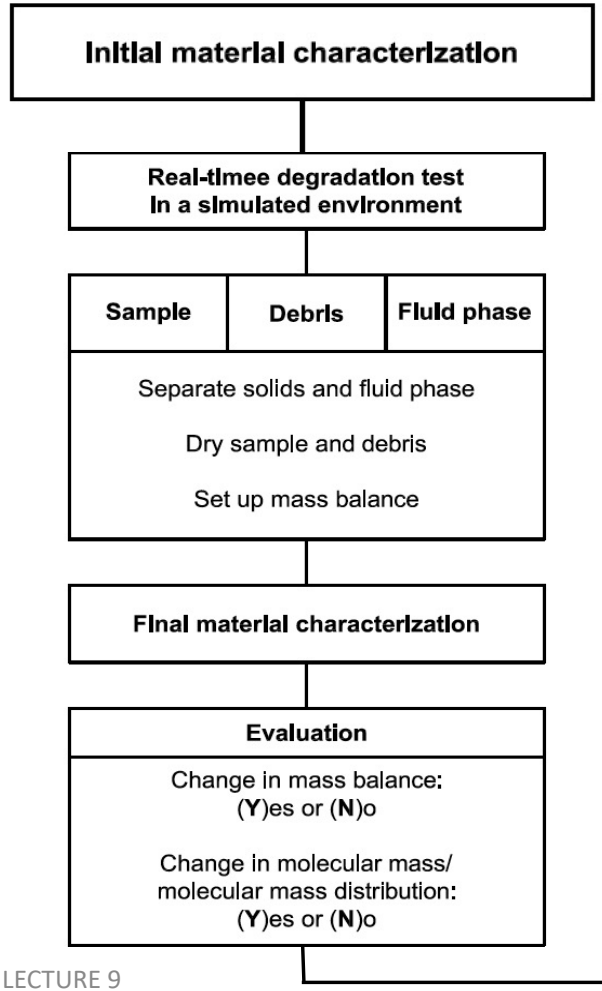
Test solutions for hydrolytic degradation

For hydrolytic degradation, the following solutions are suggested:
a) water for analytical laboratory use, grade 2, in accordance with ISO 3696;
b) buffer.

Mass/volume ratio

The ratio of the mass of the test sample to the volume of the test solution should be at least 1 g:10 ml. The samples shall be fully immersed in the test solution.

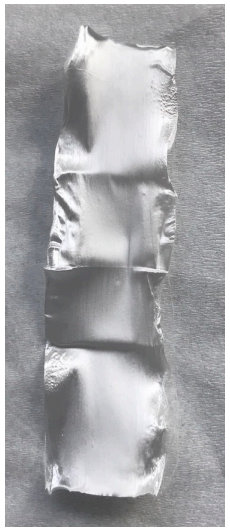
ISO 10993-13 — Part 13: Identification and quantification of degradation products from polymeric medical devices

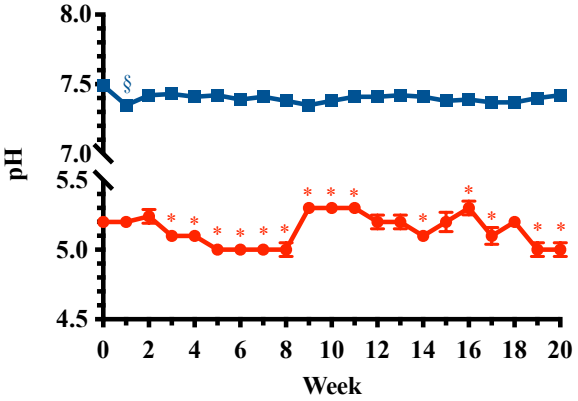


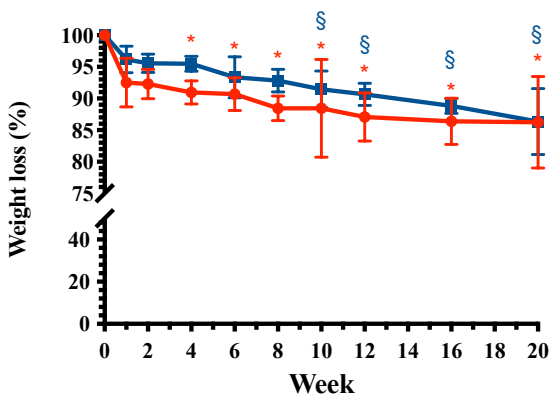
N/N	N/Y	Y/N	Y/Y
No degradation has been observed; end of test. (see NOTE in 5.4.4.2)	Check the bulk sample/debris for degradation products.	Polymer not degraded; identify and quantify leachables from the fluid phase.	Identify and quantify leachables and polymer degradation products from the fluid phase.

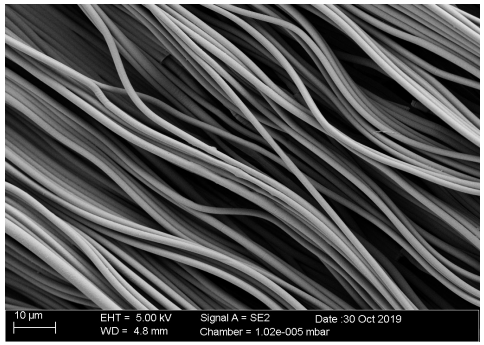
Electrospun PLGA Fleeces with highly aligned fibers subjected to hydrolytic degradation according to ISO-10993

PLGA 85:15 of LA:GA

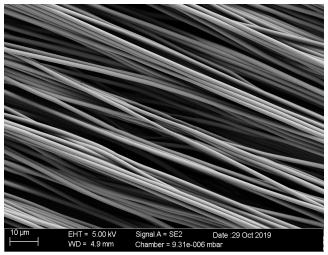


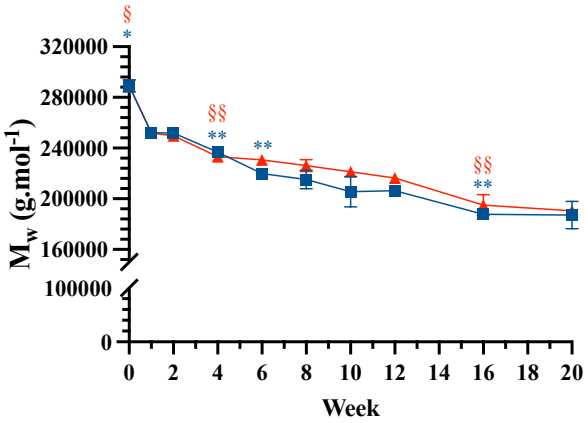


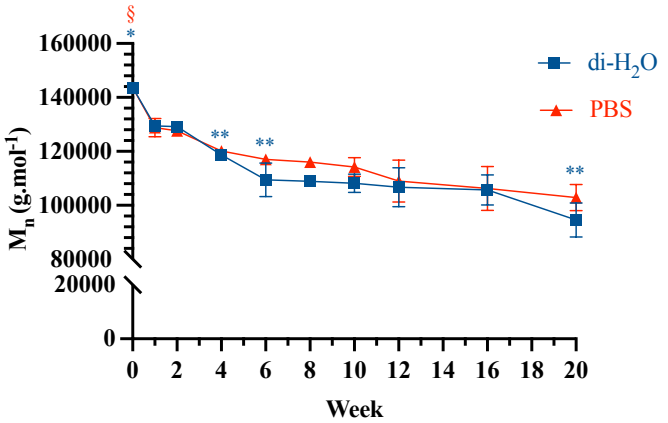


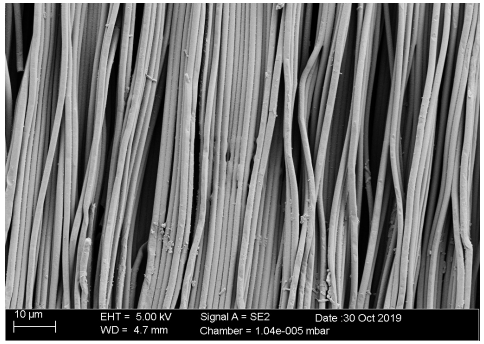


20 weeks of degradation in H_2O







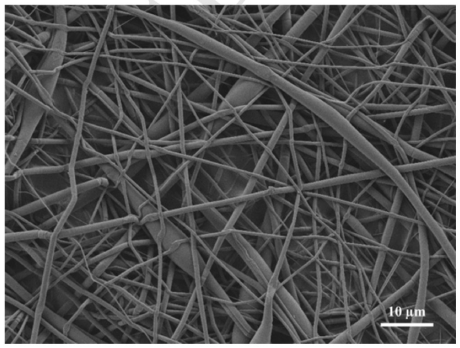


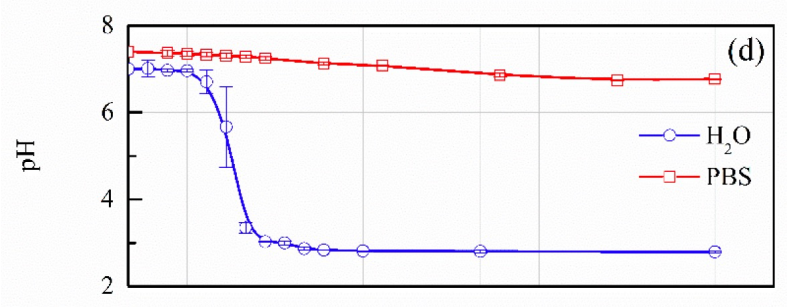
20 weeks of degradation in PBS

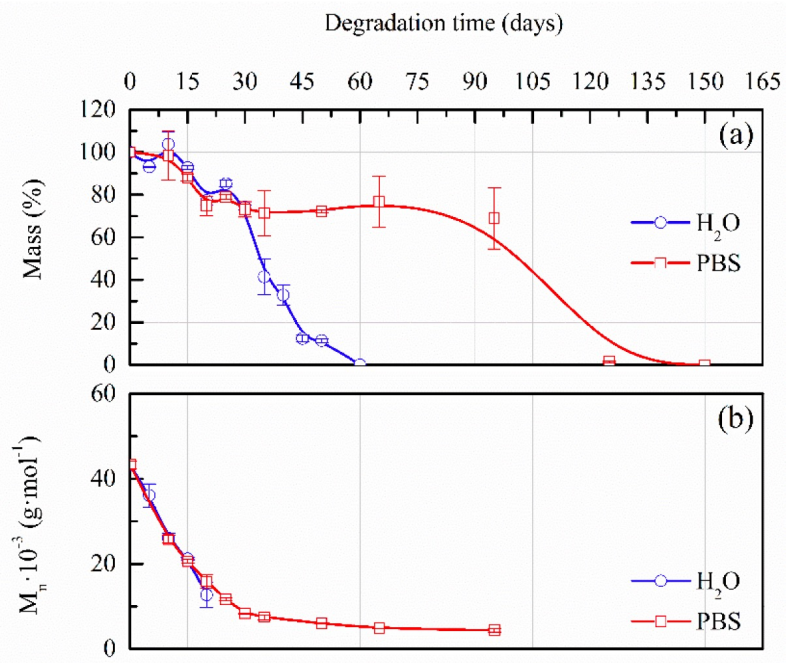
El Khatib et al., In preparation

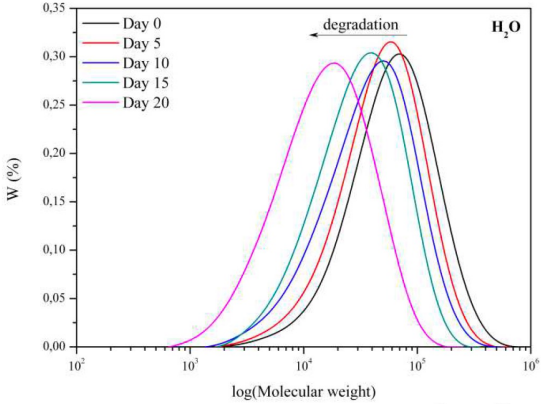
Electrospun PLGA Fleeces with highly aligned fibers subjected to hydrolytic degradation according to ISO-10993

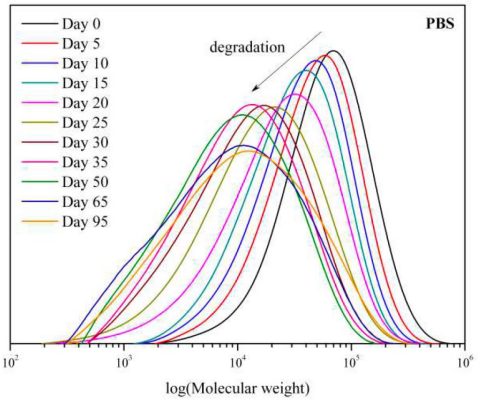
PLGA 50:50 of LA:GA











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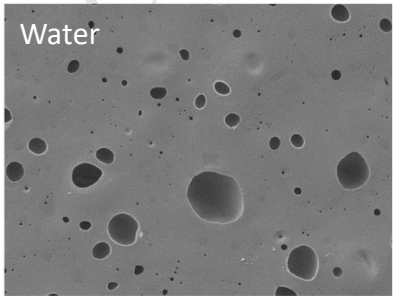

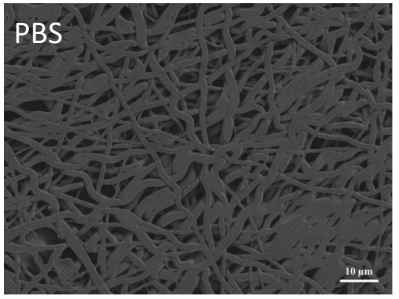

Gil-Castel, Polymer Testing Volume 66, April 2018, Pages 256-267