Nanofibrous Tissue Engineering Matrices from Poly (L-Lactide) with Improved Clinical Handling Properties for Periodontal and Craniofacial Regeneration

Seth M. Woodbury, W. Benton Swanson, Yuji Mishina
University of Michigan, School of Dentistry

Statement of Purpose: Poly (L-lactic acid) (PLLA) is widely used in tissue engineering and regenerative medicine applications, particularly as a scaffolding matrix due to its safety and biocompatibility. Nanofibrous and porous scaffolds made from PLLA have been widely demonstrated to be useful in dental and craniofacial applications. In these validation studies, the clinical defect is created to match the biomaterial, rather than having a biomaterial matrix which can fit into irregularly shaped configurations, which leaves room for improvement. While it has many advantageous properties, the clinical handling properties of many PLLA-based scaffold platforms require the ability to be shaped and maintain a shape without destroying favorable macro, micro, and nanoscale features of the material. We have developed a novel tissue engineering matrix from PLLA which allows for thermosensitive reprogramming of their macroscopic clinical shape, while maintaining micro and nano-scale features.

Materials and Methods: Nanofibrous tissue engineering matrices are prepared from high molecular weight polyesters prepared by ring opening metastasis. Thin films are made on a silica wafer, and nanofibers are induced by thermally induced phase separation. Nanofibrous, macroporous scaffolds are fabricated using a sugar sphere porogen method and thermally induced phase separation. Shape memory properties are assessed by a deformation and recovery assay protocol developed in our lab. Tensile and compressive mechanical properties are measured using an Instron mechanical tester. Scanning electron microscopy and histologic preparations are used to assess morphology at various scales. Image analysis is done in ImageJ.

Summary of the Results: Existing PLLA tissue engineering matrices fail to be molded into irregular shapes due to their strong yet inelastic mechanical properties, due to the crystallinity of PLLA. We proposed that a biodegradable thermosensitive component doped into a PLLA matrix may impart heat-induced changes in mechanical properties to allow for increased elastic deformation at elevated temperatures. We demonstrate that incorporation of a crosslinked thermosensitive switch, interpenetrated within but not covalently bound to the high molecular weight PLLA matrix, allows for heat-induced decrease in tensile modulus and increased elastic deformation. This change allows for the matrix to be conformed to an irregular shape. Importantly, this deformation does not change the nanofibrous surface morphology of our constructs resulting from the thermally induced phase separation of crystalline PLLA. At body temperature, the materials are no longer deformable, but able to maintain their new shape. Reheated to 80°C, the materials recover to their original shape.

We fabricated macroporous scaffolds from the same material, with macroporous internal structure and nanofibrous microtopography. These scaffolds appear identical to scaffolds made from PLLA, well described in the literature. We demonstrate that the compressive modulus of these scaffolds is decreased by 50% at temperatures above 80°C, increasing its elastic deformation, allowing for easy manipulation. Following a standardized deformation of 50%, we demonstrate that our novel heat-modulated matrix recovers its internal macropores *and* nanofibrous surface texture nearly completely, while PLLA scaffolds did not recover, and their internal structure was destroyed. We have demonstrated recovery of spherical macroporous and nanofibrous internal structure of these constructs in a tooth extraction model using a typodont, for proof of concept of an irregularly shaped clinical defect.

Conclusions Reached: Thermosensitive materials with a sufficiently low glass transition temperature are not crystalline, therefore unable to be fabricated with biomimetic nanofibers by thermally induced phase separation (TIPS). On the other hand, crystalline polyesters like PLLA which have favorable biologic properties and ability to form nanofibers by TIPS do not have favorable handling properties conducive for widespread clinical use in irregular defects. We have demonstrated a minimum amount of the thermosensitive component necessary for shape memory behavior, and minimum amount of PLLA necessary to maintain a nanofibrous structure. The shape memory response time is a function of both thermosensitive component molecular weight and weight percent in the mixture. Since the scaffolds are mostly PLLA, SEM data has consistently shown that they are uniformly nanofibrous and indistinguishable from our traditional PLLA scaffolds at the nanometer resolution.

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Keywords: macropore, scaffold, clinical handling, nanofiber, thermally induced phase separation