Reactive Oxygen Species-cleavable Proline Oligomer-crosslinking of Polycaprolactone for Pro-angiogenic Host Response

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Statement of Purpose: Biomaterials that exhibit specific responses to biological parameters under abnormal conditions are highly advantageous to deliver therapeutics in a spatially and temporally controlled manner. Among the biological parameters, overproduction of reactive oxygen species (ROS) is considered as a hallmark of many diseases such as cancer, atherosclerosis and inflammation. We have recently identified that a proline oligomer (KP_7K) can degrade in response to ROS[1]. Using this peptide, we have developed ROS-degradable scaffolds mainly composed of poly(ε-caprolactone)(PCL), a FDA-approved, highly biocompatible and biodegradable polymer. In this study, we aimed to optimize and validate the polymer synthesis involved and ROS-degradability of the scaffolds in a physiologically relevant condition using activated macrophages that are known to overproduce ROS. Additionally, macrophages play a key role in regulating *in vivo* host-material interactions. In particular, their phenotypic switch is an essential factor in promoting angiogenesis for successful tissue engineering. Hence, primary macrophages and their interactions with the scaffolds are investigated to determine pro-angiogenic effects of the scaffolds.

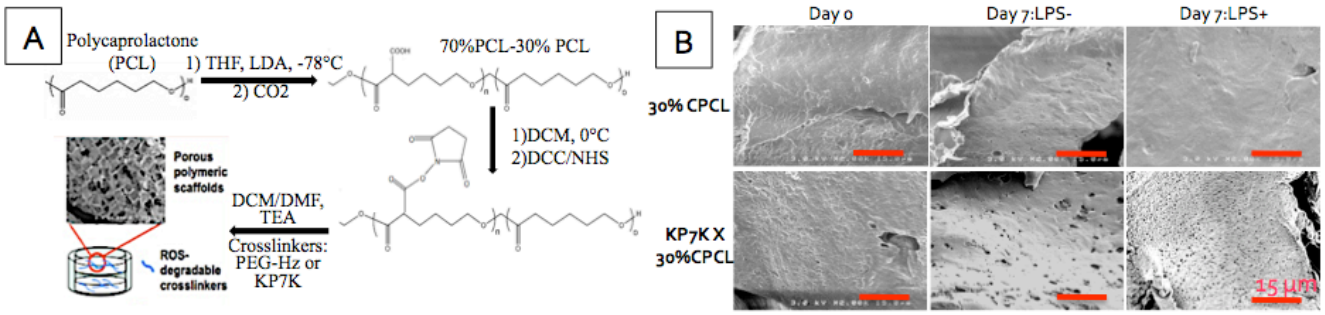
Methods: Synthesis of 70% PCL-30% carboxylated PCL(CPCL) (%: molar ratio) and fabrication of crosslinked scaffolds are demonstrated in **Fig. A**. PEG dihydrazides or KP_7K were used to crosslink the polymers. PCL and 70%PCL-30%CPCL scaffolds without any crosslinking served as controls. Polymeric scaffolds were characterized for molecular weight by GPC, composition by NMR and FTIR, thermal properties by DSC, and surface morphology by SEM. Swelling in PBS and gel content in THF were determined for crosslinked scaffolds. ROS-degradation and consequent mass changes of scaffolds were determined in treatment with 3-morpholino-sydnonimine hydrochloride (SIN-1), known to mimic a physiologically relevant ROS environment *in vitro*. For cell experiments, bone marrow-derived macrophages (BMDM) from CL57/bl6 mice were seeded on scaffolds for 10 days ±LPS. The LPS+ condition resulted in overproduction of ROS from BMDM. Gene expression of BMDM phenotypic markers were analyzed by PCR and scaffold degradation was characterized by SEM. Saffolds were implanted subcutaneously in the ventral side of mice for 2 weeks. Implanted scaffolds are examined for blood vessel formation, marker expression, and scaffold degradation.

Results: Carboxylation of PCL was done successfully to yield 70%PCL-30%CPCL by NMR with Mp of 87K and PDI of 1.4. Carboxylation alone increased the swelling ratio by 2-fold. Crosslinking 30%CPCL with hydrophilic PEG or KP_7K peptides further increased swelling in PBS by 3-fold compared to unmodified PCL. FTIR, DSC thermograms and gel content measurement confirmed successful crosslinking. To prove physiologically relevant ROS-degradation, activated BMDMs were cultured on test polymer scaffolds. SEM images showed the formation of numerous microscopic pits (<1μm) on KP_7K crosslinked scaffolds while no significant change was seen on uncrosslinked 30%CPCL (**Fig. B**). DSC and gel content measurement will be performed to further prove ROS-degradability of KP_7K crosslinked scaffolds. Next, BMDM interaction with the scaffolds *in vitro* was examined through qRT-PCR. Our preliminary data show an up-regulation of VEGF expression by 3-fold in both LPS + and – conditions on KP_7K crosslinked 30%CPCL compared to 30%CPCL. Further analyses with more samples and genes, as well as *in vivo* data analysis are currently underway and will be presented.

Conclusions: Carboxylation and NHS esterification of PCL was done sufficiently and successfully, opening a new door for functionalizing highly biocompatible and biodegradable PCL to improve its utility. We have created ROS-degradable PCL-based scaffolds by crosslinking CPCL with ROS-degradable KP_7K and characterized their chemical, physical, and thermal properties. The results show successful ROS-mediated degradation under the physiologically relevant condition. Furthermore, KP_7K cosslinked scaffolds enhanced VEGF expression, suggesting pro-angiogenic stimulation of macrophages. Other *in vitro* and *in vivo* studies are currently in progress to further elucidate macrophage interaction with ROS-degradable scaffolds and their potential pro-angiogenic effects *in vitro* and *in vivo*.

References:

1. Yu S. Biomacromolecules. 2011; 12(12): 4357-4366.



(A) Synthesis and fabrication of PCL-based scaffolds. (B) SEM images of 30%CPCL and KP7K crosslinked 70%PCL-30%CPCL scaffolds on day 0 and 7 with BMDM ± LPS activation where LPS+ condition causes ROS overproduction.