# Renewable nanoparticles as additives for 3D printed hydrogels

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Tissue engineering focuses on the *in-vitro* development of surrogates for natural tissue that help to maintain, improve or restore tissue function *in-vivo*. The use of synthetic microenvironments for *in-vitro* studies can help elucidate the molecular mechanisms of human disease and better predict the effects of drugs and therapies. Research has shown that the cellular microenvironment can dictate whether cells undergo proliferation, differentiation, apoptosis, or invasion.[1,2] In the past, most *in-vitro* studies have relied on 2D surfaces or bulk materials for cell culture and tissue engineering. The advent of technologies for 3D printing of biocompatible materials have enabled researchers to develop more sophisticated 3D architectures that could better mimic natural cell microenvironments.[3]

Early scaffold development has focused on biocompatibility issues, resulting in the predominant use of polyethylene glycol (PEG) and gelatin as the core building blocks. Although these materials offer biocompatibility, which allows for cell proliferation and differentiation, they are soft materials that in many instances cannot match the modulus of natural matrices. Therefore, attention has shifted to reinforcing such biocompatible scaffolds with nanoparticles that can confer the desired mechanical properties. Cellulose nanocrystals (CNC) are bio-based, non-cytotoxic materials that offer strength and stiffness comparable to Kevlar, and have been used in many applications as bio-additives.[4] In this study, we have used CNCs as a renewable bio-additive material for the 3D printing of hydrogel scaffolds. The scaffolds were fabricated using a two-photon polymerization direct laser writing approach where unmodified, sulfated CNCs were doped into a PEG-diacrylate (PEG-DA) matrix. We present the challenges with material preparation, protocols for PEG-DA:CNC composite production, and the optimization of conditions for the fabrication of composite scaffolds suitable for cell seeding and growth. We evaluate the scaffold quality in printing, resolution and distribution of the CNCs through SEM imaging, and evaluate the scaffold elastic modulus using nano-indentation through AFM. This work aims to introduce CNCs as non-cytotoxic bio-based additives for 3D printing resins that enhance the stiffness and confer sites suitable for cellular adhesion. The use of cellulose as an additive also opens the door for the future chemical modification of the scaffolds.

**References:**

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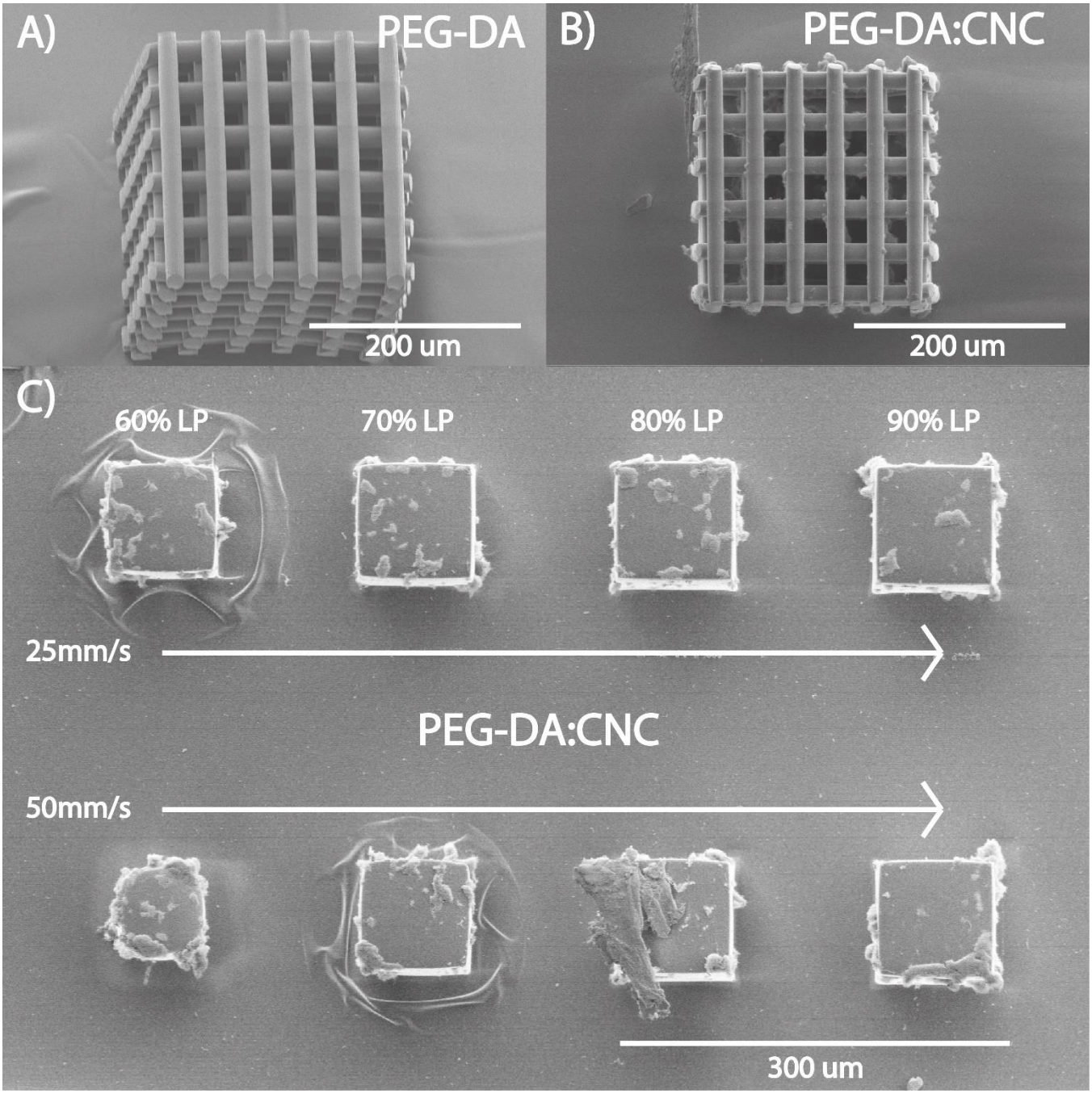


**Figure 1.** SEM images of 3D printed scaffolds using Nanoscribe. a) A neat PEG-DA woodpile scaffold serving as a control for comparison to CNC doped PEG-DA prints. b) A CNC doped PEG-DA scaffold, demonstrating comparable resolution prints to neat PEG-DA. This shows that by adding CNCs, we are able to introduce non-cytotoxic biomaterials to pre-existing bulk printing materials without ruining the print quality. c) PEG-DA:CNC cubes (100x100x100 µm) printed with different LASER powers (LP) and write speeds (WS).