Nanocellulose Targets Regenerative Medicine

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The term “nanocellulose” encompasses a wide range of cellulose-derived nanomaterials, which are characterized by their diverse morphologies and chemical structures at the nanometer scale. In recent years, there has been a remarkable increase in the use of nanocellulose in regenerative medicine. Of particular interest is the *in vitro* culture of pluripotent stem cells, which has led to a growing demand for animal-free media and scaffolds in stem cell culture. Extracellular matrix (ECM) plays a pivotal role in regulating the proliferation and differentiation of stem cells, with fibrous collagen and glycosaminoglycans serving as structural and functional supports. In this context, nanocellulose has garnered significant interest as potential ECM mimetics, due to its rigid fiber form and its regular interfacial structures. The use of natural polysaccharide nanofibers would represent a promising avenue for the functional design of animal-free cell culture scaffolds, with the potential to significantly advance the regulation of stem cell culture in regenerative medicine.

DOI: 10.15376/biores.20.2.2464-2467

Keywords: Cellulose nanofiber; Surface modification; Extracellular matrix mimetics; Xeno-free; Stem cell

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Essential Architectures of Extracellular Matrix

Tissue engineering and regenerative medicine offer strategies to promote human health and longevity. There is a need to develop biomedical substitutes that maintain or repair tissue functions or whole organs. Extracellular matrix (ECM) mimetics hold a key to realizing *in vitro* cell culture and organ constructs. This is because most of our cells are surrounded by the ECM components such as collagen, fibroin, and glycosaminoglycans (GAGs) to form tissues and organs *in vivo* (Mehvari *et al.* 2024). In this context, the paradigm shift from conventional synthetic polymers to advanced biomaterials offers new opportunities for organ transplantation and tissue repair. Synthetic materials with excellent designability and well-defined chemical compositions certainly allow precise control of mechanical and degradation properties; however, these artificial objects bear no resemblance to natural ECM components, which imposes limitations. Natural biopolymers such as algal polysaccharides have some similarity; however, their structures are often ambiguous due to impure mixture and difficulty in isolation, resulting in significant batch-to-batch variation that is uncontrollable in practical applications.

In recent years, structural polysaccharides such as cellulose nanofibers (CNFs) derived from woody bioresources have attracted much interest due to their well-defined nature. Designed CNFs with different surface chemistries possess essential ECM-like architectures for both rigid fiber morphology similar to structural proteins, such as collagen and regular GAG-like chemical structures. Therefore, engineered CNFs are the most promising natural nanomaterials, which are expected to be used in biomedical applications rather than synthetic polymers and other natural crude polysaccharides. In addition, CNFs

naturally contain no animal-derived components, which is advantageous for use as a novel medical modality in regenerative medicine from the perspectives of avoiding immune rejection, quality control such as lot-to-lot differences, and animal health.

Recent Advances in Cell Culture on Surface-modified CNF Scaffolds

Cellulose is an inherently bioinert material that repels the adsorption of proteins, which is advantageous for dialysis membranes. Similarly, CNFs exhibit poor bioadaptability as cell culture scaffolds. Recently, tailoring the surface chemistry of CNFs by introducing functional groups, such as carboxy (carboxylate), sulfate, phosphate, or amino groups, has been found to significantly improve their applicability as bioadaptive scaffolds (Kamdem Tamo 2024). TEMPO-oxidized CNFs (TOCNFs) with cellulose I crystalline structures have been shown to facilitate optimal cell adhesion and proliferation of mouse fibroblasts and myoblasts in a carboxylate-content-dependent manner (Hatakeyama and Kitaoka 2022). Neither carboxylate-free original CNFs nor carboxylate-rich TOCNFs can contribute to cell growth. In addition, osteoblast-like cells proliferate well, leading to osteogenic differentiation and calcification, even in the absence of a differentiation inducer, which highlights the potential in bone tissue engineering. Human liver cells can be cultured in porous 3D scaffolds and bioink gels for 3D printing using TOCNFs and chitosan NFs. Surface-deacetylated chitin NFs with chitosan structure on their surface are also bioinert, but the combination with TOCNFs significantly promotes the skin repair for wound healing. A variety of surface-modified CNFs and their structural analogues, such as chitin and chitosan NFs, exhibit the strategies to tune their bioadaptability by introducing different functional group moieties, where the ECM-mimetic morphology and interfacial structures would hold the key.

Human Stem Cell Culture under Completely Xeno-free Conditions

Inspired by the extensive applications of sulfated glycosaminoglycans in the human ECM *in vivo*, the introduction of carboxy and sulfate groups into bioinert CNFs has attracted considerable interest in recent biomedical research. In particular, the combination of TOCNFs and sulfated CNFs (S-CNFs) allows the efficient culture of primary human mesenchymal stem cells (hMSCs) under completely xeno-free conditions, meaning that the culture system does not contain any animal-derived components in either the scaffold or the culture medium (Kai *et al.* 2024). As shown in Fig. 1, iliac bone-derived hMSCs are efficiently cultured on surface-modified CNF scaffolds while maintaining the inherent multipotency of hMSCs, which is essential for practical stem cell therapies. Both carboxy and sulfate content of surface-modified CNFs are the key to regulating the hMSCs culture. The TOCNFs (COONa: 1.47 mmol/g; fiber length: 0.53 μm), the S-CNFs (OSO_3Na: 0.64 mmol/g; 0.61 μm), and a combination of both (1:1 by weight) enable primary hMSCs to proliferate, comparable to animal-derived type I collagen scaffolds in a serum-free medium. Plant-derived nanomaterials without immunological rejection may become a game changer in regenerative medicine.

From another perspective, heparin-mimicking sulfated carboxymethylcellulose conjugated to modified gelatin provides another approach for regenerative medicine through preferentially binding to proteins and heparin-binding growth factors, including TGF-β1, CTGF, and FGF-2 (Bhutada *et al.* 2021). Immobilization of growth factors on cell culture scaffolds would significantly improve their long-term stability and bioactivity, which are expected to contribute to cell proliferation and differentiation. The functional

design of sulfated polysaccharide NFs has great potential for tissue engineering and regenerative medicine due to their diverse array of bioactivity *in vivo*.

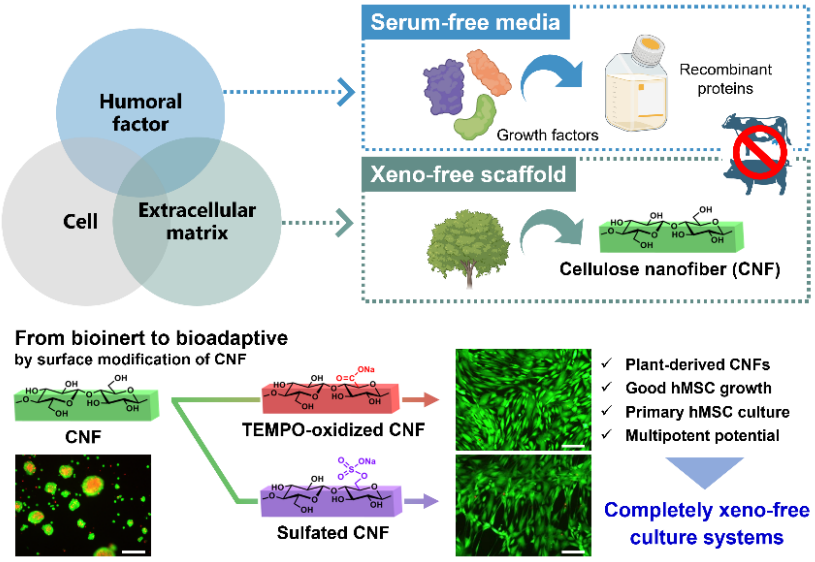

Fig. 1. Schematic research strategy of animal component-free cell culture scaffolds composed of TEMPO-oxidized CNFs (TOCNFs) and sulfated CNFs (S-CNFs), on which primary human mesenchymal stem cells (hMSCs) are cultured under serum-free conditions. Scale bars correspond to 200 µm. Reprinted under the terms of the CC-BY 4.0 license (Kai *et al.* 2024), Copyright 2024, Elsevier.

Future Opportunities

Surface modification strategies of nanocellulose with ECM-like morphologies and chemical structures have great potential for designing functionalities of biomedical CNFs. Based on physiological anatomical findings, phosphorylated CNFs may be promising for human dental pulp stem cell culture for tooth regeneration and neural differentiation for nervous system repair. Pioneering work on the emerging functions of nanocellulose as a new medical modality in regenerative medicine will signal a new trend to advance medical nanocellulose industry, in line with Sustainable Development Goals (SDGs) #3 and #15.

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