ABSTRACT

Since the last three decades, extensive efforts have been undertaken towards the regeneration and/or replacement of damaged or diseased organs by tissue engineering. However, there have only been a sporadic success with organ transplantation regarding the development of healthy tissues such as cartilage, bone, skin, cornea, etc., owing to the intricacy of the human tissues. Furthermore, it is acknowledged that developing designed organs and tissues for transplantation is likely complex because our knowledge of organ regeneration and related technologies is still minimal. The conventional tissue engineering methods fail to configure the desired tissues with intricate biomimetic architecture. Three-dimensional (3D) bioprinting, also known as 3D bioprinting, is highly promising that overcomes the constraints of traditional tissue engineering practices. The advantage of 3D bioprinting is that it permits the development of layer-by-layer custom-made, patient-specific constructs by fabricating anatomically relevant implants akin to the complexity of the native tissue using the cells from the patient itself. In light of this, the present study highlights the application of 3D bioprinting and decellularization techniques for soft and hard tissue regeneration, explicitly focusing on cartilage, bone, skin, and cornea.

Most of the articular cartilage is comprised of a collagen II fiber network arranged in arches. The lack of suitable microfabrication techniques capable of developing 3D fibrous structures is one of the biggest obstacles to *in vitro* replication of cartilaginous tissue with an arch-like structure. As a result, in contrast to the conventional wood-pile pattern, we provide in the first study a 3D bioprinting technique for producing constructs that resemble the arches of collagen II using two different types of bioinks: gelatin methacryloyl (GelMa) and silk fibroin-gelatin (SF-G). SF-G bioink laden with mesenchymal stem cells derived from human bone marrow (BM-MSCs) exhibited its prominent role compared to GelMA in the fibrous collagen network formation and chondrogenesis as ascertained from the biochemical assay, gene, and protein expression. This was further validated by a protein-protein interaction study using Metascape, GeneMANIA, and STRING analysis. These findings demonstrated the role of SF in the modulation of Wnt/β-catenin and TGF-β signaling pathways. Therefore, the arch-like 3D bioprinted constructs exhibit a significant promise for articular cartilage regeneration.

In our second study, we developed a shear-thinning bioink multicomponent in nature composed of Alginate and Gellan Gum with acceptable mechanical and printing capabilities. The bioink was reinforced by adding two kinds of silk nanoparticles (SNP), i.e., native and regenerated, improving the printability. The bioink with the SF solution acts as a control set.

The rheological and mechanical properties of the Alginate-Gellan Gum bioink were enhanced by the addition of native SNP. However, the control group exhibited better chondrogenesis with BM-MSCs as determined by the RT-PCR, immunofluorescence, and histology analysis. Hence, adding the SNP by us improved the overall printability while maintaining the chondrogenesis of articular cartilage.

Hydroxyapatite (HAP) shows promising applications for treating bone abnormalities. However, traditional foaming procedures could minimally regulate the pore size, geometry, and interconnectivity of 3D porous HAP scaffolds, which limits their clinical success. It is challenging to replicate the complex 3D framework and the effective dynamics of skeletal distortions, emphasizing the need for a personalized, coveted tissue substitute, for which 3D printing offers an alternative. Hence, the third study describes the fabrication of a 3D-printed HAP scaffold and their potential for *in vivo* bone regeneration in tibial defects of rat. Micro-computed tomography (micro-CT) and histomorphometry analysis were used to determine bone ingrowth. Implants at the defect site showed significant bone ingrowth. Micro-CT measurements revealed that the implant's bone mineral density (BMD) at the defect site was 1024 mgHA ccm^{-1}, which was almost 61.5 percent greater than the BMD at the sham control site. The hematoxylin and eosin micrographs also showed no sign of any immunoinflammatory response. The findings imply that the 3D-printed HAP scaffolds provided a sufficient matrix for producing patient as well as defect-specific bone growth.

Developing an *in vitro* aging skin model is challenging since aging is a complex activity throughout a person’s lifetime. Moreover, skin is composed of more than a single cell type; hence it is challenging to replicate this composite phenomenon within a short time. Therefore, in our fourth study, we describe the development of a 3D bioprinted skin aging model utilizing SF-G bioink, recapitulating the dual layer of the dermis and epidermis present in native-aged skin. We have exploited the use of the senescent or late passage fibroblast cells, keratinocytes, and melanocytes in co-culture. This was compared with the early passage cells as a control. Reduced fibronectin expression in gene and protein analysis accompanied by a decline in the expression of collagen I, II, and IV demonstrate the potency of our 3D bioprinted model equivalent in recapitulating the attributes of extrinsic aging *in vitro*. Further, we developed two pathological conditions: oxidative stress induced by H_2O_2 and high glucose as other prospective alternative agents in inducing senescence. Our developed 3D bioprinted skin aging model has numerous uses in basic research on aging, disease modeling, and the screening of active pharmaceutical ingredients.

Decellularized corneas derived from different species have become extremely popular in tissue engineering due to donor tissue scarcity. The decellularized cornea is discovered to elicit an immune reaction despite removing the cellular constituents and antigens since the collagen fibrils are distorted, exposing specific antigenic regions that frequently result in transplant rejection. Hence, our final study highlights the modulation of decellularized corneas from goats using chondroitin sulfate and their implantation into rabbit stroma, citing the distorted collagen conformation getting restored. Healthy monocytes and differentiated macrophages were studied with their surface marker in an *in vitro* immune response study using pHrodo red, Lysotracker red, ER tracker, and CD63, LAMP-2 antibodies that established that when compared to decellularized matrices, cross-linked ones produced the weakest immunological response. Further, histological staining after three months of implantation *in vivo* into the stroma of rabbits validated our *in vitro* findings. As a result, we conclude that the chondroitin sulfate cross-linked to the decellularized corneal matrix may function effectively as a replacement for allograft and human cadaveric corneas.