Isotropic and Anisotropic Cryogel Matrices for Cartilage Tissue Engineering

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Introduction: O*steoarthritis,* a degenerative joint disease associated with pain in joints, affects approximately 9.6% of males and 18% of females aged ≥60 years worldwide. Recent survey has shown that osteoarthritis is world's abreast ailment affecting the adults, estimated 20.7 million only in US. Tissue engineering holds great promise for the generation of functional tissue substitutes like cartilage, by engineering tissue construct *in vitro* for successive implantation *in vivo.* Since two decades *Cryogels* have been successfully utilized for many biomedical and bioengineering applications. Recent studies attract its use for tissue engineering applications. Our aim is to employ tissue engineering approach to repair the damaged cartilage by seeding of primary chondrocytes on biocompatible polymeric cryogel scaffolds to treat osteoarthritis. We have previously developed several novel cryogel matrices with isotropic and anisotropic properties like agarose-gelatin, gelatin-alginate and agarose-alginate. These cryogels have high interconnected pores and porosity, amiable mechanical integrity and natural ECM like properties, which is being utilized for neo-cartilage development.

Methods: Cryogel matrices have been developed by *cryotropic gelation* of natural polymers at subzero temperature, which leads to the formation of 3D interconnected porous network. Further, the primary chondrocytes were isolated from goat knee and drop-wise seeded in these matrices. The seeded chondrocytes were cultured for 7 to 8 weeks in DMEM growth medium at 37°C in humidified environment with 5% CO_2. The preliminary studies were performed to analyze the growth of chondrocytes and natural ECM production by them in cryogels using microscopic and biochemical analysis.

Results: The increasing growth and proliferation of chondrocytes in cryogel matrices was observed by DAPI nuclear staining which was further confirmed by cell proliferation assay i.e. MTT to determine the cellular activity of chondrocytes. The seeded cells were found highly active and were continuously secreting extracellular matrix to their surrounding environment of cryogel. The observations suggest that the secreted component of ECM has less dense microenvironment and flabby in nature which caused the upward movement of ECM matrix in culture medium and leads its accumulation on the cryogel matrix, which could be later separated from cryogel matrix by applying physical forces as intact tissue body (Figure 1). Although, the cryogel matrix were also covered with ECM secreted by cells in their middle portion along with the abundant cell population observed by SEM analysis. The growth pattern of cell was found distinct in agarose and alginate cryogels where cells were more likely to grow in clusters while chitosan and gelatin cryogels allowed uniform cell growth. It might be due to the masking nature of agarose and alginate, which are chemically inert by nature and don't affect the cellular physiology and help to maintain-

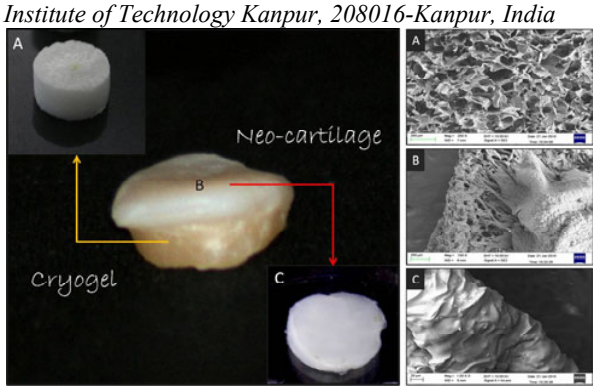

Figure 1. Neo-cartilage development in cryogels. The digital images show the cryogel matrix (A), on which the neo-cartilage was developed (B) and later intact neo-cartilage was manually separated from cryogel (C). The SEM images in other panel demonstrate microscopic view of A, B and C, respectively.

-chondrocytes phenotype. Furthermore, the comparative studies were performed to access the similarities of neo-cartilage to the native cartilage. The cryo-sectioned thin micro-sections of neo-cartilage stained with DAPI showed native cartilage (control) like cells distribution while the DNA quantification analysis suggested that the amount of total DNA was significantly similar in both the tissues.

Conclusions: The cryogels used in this study were developed using natural polymers, which facilitates the biocompatible and biodegradable properties to these matrices. Up to 90 % porosity and high nutrient, cells and waste transport makes them attractive class of future biomaterials. Our studies also showed high elastic properties of these cryogels, which could facilitate good mechanical signals to the cells during stress-strain. Overall, the present finding of high amount of ECM secretion and cellular activity of cartilage cells in cryogel matrix is suggesting its potential utilization for neo-tissue development. The cryogels could be used as a support structure for *in vitro* neo-cartilage development followed by implantation at the damaged site in the body. On the other hand, we believe that the biodegradable cryogels along with cells could be implanted where the cryogel matrix will be replaced by the cell secreted ECM with the time. This study rationalized the successive use of macroporous polymeric cryogels for *in vitro* neo-cartilage development using natural polymers. Although, the further process characterization are required for the improvement of neo-cartilage like mechanical stiffness, which could be a future direction in this field.

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