World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:10, No:05, 2016

D-Lysine Assisted 1-Ethyl-3-(3-Dimethylaminopropyl)Carbodiimide / N-Hydroxy Succinimide Initiated Crosslinked Collagen Scaffold with Controlled Structural and Surface Properties

Authors : G. Krishnamoorthy, S. Anandhakumar

Abstract : The effect of D-Lysine (D-Lys) on collagen with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide(EDC)/N-hydroxysuccinimide(NHS) initiated cross linking using experimental and modelling tools are evaluated. The results of the Coll-D-Lys-EDC/NHS scaffold also indicate an increase in the tensile strength (TS), percentage of elongation (% E), denaturation temperature (Td), and decrease the decomposition rate compared to L-Lys-EDC/NHS. Scanning electron microscopic (SEM) and atomic force microscopic (AFM) analyses revealed a well ordered with properly oriented and well-aligned structure of scaffold. The D-Lys stabilizes the scaffold against degradation by collagenase than L-Lys. The cell assay showed more than 98% fibroblast viability (NIH3T3) and improved cell adhesions, protein adsorption after 72h of culture when compared with native scaffold. Cell attachment after 74h was robust, with cytoskeletal analysis showing that the attached cells were aligned along the fibers assuming a spindle-shape appearance, despite, gene expression analyses revealed no apparent alterations in mRNA levels, although cell proliferation was not adversely affected. D-Lysine (D-Lys) plays a pivotal role in the self-assembly and conformation of collagen fibrils. The D-Lys assisted EDC/NHS initiated cross-linking induces the formation of an carboxamide by the activation of the side chain -COOH group, followed by aminolysis of the O-iso acylurea intermediates by the -NH2 groups are directly joined via an isopeptides bond. This leads to the formation of intra- and inter-helical cross links. Modeling studies indicated that D-Lys bind with collagen-like peptide (CLP) through multiple H-bonding and hydrophobic interactions. Orientational changes in collagenase on CLP-D-Lys are observed which may decrease its accessibility to degradation and stabilize CLP against the action of the former. D-Lys has lowest binding energy and improved fibrillar-assembly and staggered alignment without the undesired structural stiffness and aggregations. The proteolytic machinery is not well equipped to deal with Coll-D-Lys than Coll-L-Lys scaffold. The information derived from the present study could help in designing collagenolytically stable heterochiral collagen based scaffold for biomedical applications.

Keywords : collagen, collagenase, collagen like peptide, D-lysine, heterochiral collagen scaffold
Conference Title : ICTERMA 2016 : International Conference on Tissue Engineering and Regenerative Medicine Applications
Conference Location : Amsterdam, Netherlands
Conference Dates : May 12-13, 2016