Supplementary File (S1)

Biodegradability assay

Introduction

In order to study the application of cross-linked collagen biopolymer materials as implants, it is necessary to assess the biodegradability of the biopolymer developed. Since, numbers of enzymes were produced upon various biochemical reactions inside the body, degradation studies using enzymes found reliable.

Experimental details

In the present study, we follow a procedure to assess the biodegradability of CFNGO biopolymer material. The procedure demonstrates the measurement of release of hydroxy proline by the enzymatic treatment of CFNGO and native collagen[1]. In brief, in the hydroxy proline assay method, reaction mixture containing 10 mg of samples (CFNGO and native collagen) along with 0.9 ml of 20 mM Tris-HCl (pH 7.5) and 0.1 ml of the enzyme solution was incubated at 37° C for different time intervals. At schedule time points, reaction was stopped by the addition of 0.2 ml of 50% trichloroacetic acid and incubated at room temperature for 10 min and centrifuged at 6000 rpm for 20 min. From the supernatant 100 - 200 µl of supernatant was mixed with 1.0 ml of chloramine T and incubated at room temperature for 20 min and 1ml of perchloric acid (70%) was added and the incubation continued at room temperature for 5 min and then 1ml of 20 % PDAB (Para- dimethyl amino benzaldehyde) was added and incubated at 60°C in water bath for 20 min and the absorbance of the resulting solution was measured at 557 nm.

Results and Discussion

CFNGO and native collagen degradable in the presence of collagenase enzyme with in the period of 4- 20 h of incubation as assessed in terms of release of hydroxy proline (Fig. 1). When compared to native collagen material, the release of hydroxy proline was significantly less ($P<0.05$) in the cross-linked CFNGO material.

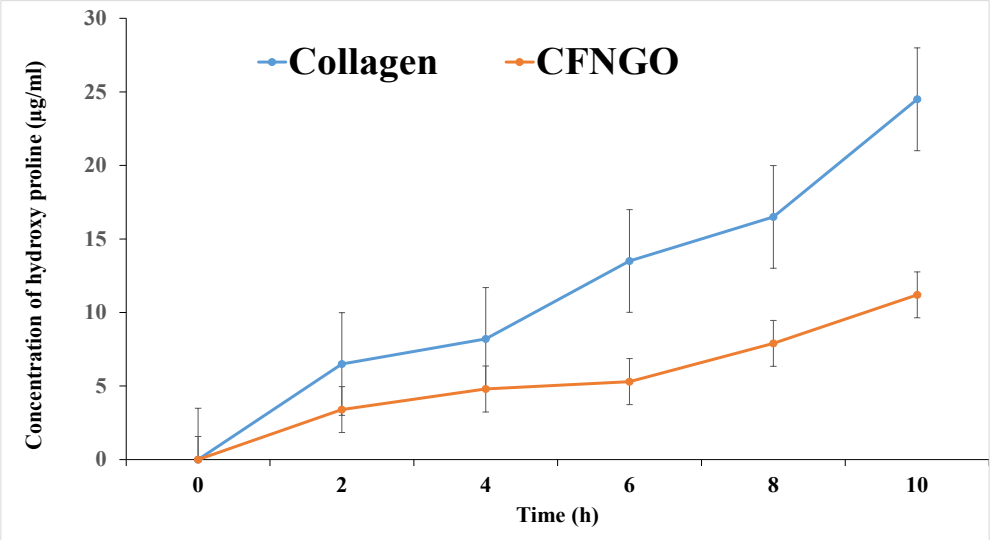

Fig.1. Release of hydroxy proline from collagen functionalized nano graphene oxide (CFNGO) and native fish scale collagen.

The enzymatic degradation results of CFNGO and collagen depicted both the material degraded when treated with collagenase enzyme. However, the rate of release of hydroxy proline showed significant difference compared with native collagen. In native collagen, release of hydroxy proline starts from the minute of exposure to enzymes compared to cross-linked collagen. Thus, the results of the study showed the cross-linked CFNGO biopolymer material is biodegradable.

Reference

1. G.K. Reddy and C.S. Enwemeka, *Clinical Biochemistry,* 1996, **29**, 225-229.