# Control of Biodegradation of Chitosan/Gelatin Scaffolds for Tissue Engineering

**(Proof of Principle Study)**

Thesis

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# *Dedication*

*This work is dedicated to the soul of my beloved brother Adham. I wish you were here with that look on your face that makes me feel proud.*

*My mother, my hero, my survivor, couldn't have made it without you.*

*My greatest blessing, my son Mallek, my daughter Jamila and my beloved husband.*

*Last but not least, my father and sister I'm so grateful.*

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***List of abbreviations:***

| Name | Abbreviation |
|---|---|
| Tissue engineering | TE |
| Three dimensional | 3D |
| Pondus hydrogenii or potential hydrogen | pH |
| Chitosan | C |
| Gelatin | G |
| Degree of deacetylation | DDA |
| Hydroxyapatite | H |
| β-tricalcium phosphate | β-TCP |
| Biphasic calcium phosphate | BCP |
| Synthetic hydroxyapatites | sHA |
| Thermal gravimetric analysis | TGA |
| Nano hydroxyapatite | nHA |
| Human mesenchymal stem cells | hMSCs |
| Hematoxylin and eosin | H&E |
| Polycaprolactone | PCL |
| Food and drug administration | FDA |
| Poly L-lactic acid | PLLA |
| Poly(L-lactide-*co*-D,L-lactide) | PLDLLA |
| Poly-lactic-co-glycolic acid | PLGA |
| Polyglycolic acid | PGA |
| Polyethylene glycol | PEG |
| Polybutylene terephthalate | PBT |

| | |
|---|---|
| Polyurethanes | PU |
| polyhedral oligomeric silsesquioxane | POSS |
| Glycosaminoglycans | GAGs |
| Ultraviolet radiation | UV |
| Thermally induced phase separation | TIPS |
| arginine–glycine–aspartic acid | RGD |
| Computer-assisted design | CAD |
| Computed tomography | CT |
| Dehydrothermal | DHT |
| Riboflavin and ultraviolet-A | RFUVA |
| Transglutaminase | TGase |
| Hydrogen peroxide | H2O2 |
| Glutaraldehyde | gl |
| Genipin | gp |
| 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide | EDC |
| Citric acid | CA |
| Mineral trioxide aggregate | MTA |
| Dibasic sodium phosphate | DSP |
| Scanning electron microscopy | SEM |
| Atomic force microscope | AFM |
| Simulated body Fluid | SBF |
| Bone morphogenic protein | BMP |
| Gram | gm |
| Kilo Newton | kN |
| Dalton | Da |
| Hour | H |

# Introduction

Every day thousands of surgical procedures are performed to replace or repair tissues that have been damaged through disease or trauma. The developing field of tissue engineering aims to regenerate damaged tissues by combining cells from the body with highly porous scaffold biomaterials, which act as templates for tissue regeneration, to guide the growth of new tissue. [1]

The biological, chemical, and architectural requirements of the materials utilized in the manufacture of scaffolds are biocompatibility, biodegradation, adequate mechanical resistance, three-dimensional structure, uniformly interconnected pores, and the ability to be mold into different shapes or dimensions. Under suitable conditions, tissue repair is favored by the bioactive characteristics of the material(s) that make up the scaffold. [2-3]

From different materials used in fabrication of scaffolds chitosan and gelatin have been studied and applied commonly to form porous sponges that render good infiltration to the cells at the implantation site. [4]

They are commonly blended for use in bone tissue engineering as this blend offers the structural similarity of chitosan to the extracellular matrix and the lower antigenicity of gelatin in comparison to collagen prior to its low cost. In addition, gelatin also retains some information signals, such as arginine–glycine–aspartic acid (RGD), which is conducive to the acceleration of cell differentiation, proliferation, and attachment to materials.[5]

Both chitosan and gelatin are biodegradable, biocompatible, and nontoxic, and they are beneficial for cell attachment and proliferation but

they lack the bioactivity offered by the combination of inorganic particles such as hydroxyapatite which can further facilitate the repair of bones. Furthermore, they both have water as their basic solvent, and this benefits freeze-drying preparation.[6]

However, the physical and mechanical stability of this blend in aqueous solutions is limited and crosslinking agents are required to increase their performances in a biological environment to offer a chance for the new bone to be formed and act as a mechanical support to tissues.[7]

Glutaraldehyde and genipin are two crosslinking agents that are commonly used with chitosan and gelatin. The amines of chitosan and gelatin can be formed into amine–amine bonds via crosslinking with gluteraldehyde and genipin enhancing their degradation rate. With the gluteraldehyde that is well known to be a strong crosslinker despite its potential cytotoxicity , and the genipin being a natural crosslinker that is safe on tissues we might be able to tailor the degradation rate of the scaffold.[8]

Despite the numerous reports on the potential applications of chitosan based scaffolds as biomaterials in clinical and the pharmaceutical field, reports on control of biodegradation that compare the effect of different crosslinkers are still lacking. [9]

For this reason in this work we were aiming to control the rate of degradation without compromising the physicochemical, thermal, mechanical properties of the scaffolds even after the incorporation of bioactive materials and affecting its role in cell proliferation.