Topic: Biomaterials
Subtopic: Hydrogels

TERMIS25_129 - Reinforced multifunctional gellan gum hydrogels with nano-hydroxyapatite and carbon nanotubes for improved bone regeneration

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Introduction/Objectives

Bone can heal minor damage naturally, but larger defects often lead to scar tissue or weaknesses. Hydrogels offer an injectable, biocompatible alternative to grafts, capable of delivering bioactive cues and cells to defect sites. This study aimed to develop a multifunctional biomimetic material for minimally invasive bone regeneration. Gellan gum methacrylated (GGMA) hydrogels were reinforced with carbon nanotubes (GGMA-CNT) to mimic collagen fibers, while nano-hydroxyapatite (nHAp) was synthesized in situ on the CNTs (GGMA-nHApCNT), mimicking the natural self-assembly of hydroxyapatite on collagen.

Methods

GGMA was synthesized by reacting low-acyl gellan gum with glycidyl methacrylate. In situ nucleation of nHAp on CNT was promoted by Ca^{2+} interacting with COO^- groups on CNT, followed by PO_4^{3-} addition. Methacrylation was confirmed by NMR, and nHApCNT was characterized by Confocal Raman spectroscopy, XRD, XPS, and wettability tests. To mimic bone matrix, GGMA was mixed with nHApCNT. Rheological, structural, bioactivity, antioxidant, antimicrobial, and hemolytic properties were evaluated. Human adipose-derived stem cells were encapsulated in the hydrogels and cultured for 21 days under basal and osteogenic conditions. Cell viability, proliferation, morphology, metabolic activity, alkaline phosphatase (ALP) activity, and gene expression (OP, Colg I, ALP, BSP) were analyzed.

Results

Methacrylation was confirmed by NMR, and the presence of nHAp on CNT was validated using Confocal Raman spectroscopy, XRD, and XPS. Mechanical analysis revealed that CNT disrupted the hydrogel matrix due to their hydrophobicity, reducing mechanical stability. However, the incorporation of nHApCNT restored stability to levels similar to GGMA hydrogels. Bioactivity assays confirmed mineralization in all samples, with GGMA-nHApCNT hydrogels showing significantly higher calcium content after 21 days. All hydrogels exhibited antioxidant activity. Hemocompatibility tests revealed 5.8% hemolysis in GGMA-CNT hydrogels, indicating minor red blood cell rupture, but none in GGMA-nHApCNT and GGMA hydrogels. Encapsulated cells were viable over 21 days, with increased cell spreading in GGMA-nHApCNT hydrogels. DNA quantification confirmed cell proliferation, with greater growth in GGMA-nHApCNT compared to GGMA or GGMA-CNT. Cells remained metabolically active, with higher rates in GGMA and GGMA-nHApCNT hydrogels than in GGMA-CNT. ALP activity peaked at day 14, particularly in GGMA-nHApCNT hydrogels, indicating nHAp's role in osteogenesis. Gene expression showed higher levels of Colg I, ALP, and OP in GGMA-nHApCNT hydrogels, though BSP expression was lower compared to GGMA-CNT.

Conclusions

The reinforced GGMA-nHApCNT hydrogels showed enhanced mechanical stability, bioactivity, and osteogenic potential compared to GGMA-CNT hydrogels. Incorporation of nHAp reinforced the structure, promoted mineralization, and improved cell proliferation and differentiation. Cell viability were maintained, with no hemolysis and increased osteogenic marker expression. These hydrogels offer a promising platform for minimally invasive bone regeneration applications.

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