Conjugation of CTGF with Reduced Graphene Oxide Nanoparticles for the development of wound healing hydrogel patch

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Abstract

- Non-healing chronic wounds are the key concern in type-2 diabetes that frequently leads to chronic infections, finally causes amputation of limbs, organs etc..
- Decrease in the proliferation and migration of cells such as keratinocytes and fibroblasts is the major reason for the development of such chronic diabetic wounds.
- Multiple evidences have shown that CTGF and reduced graphene oxide possesses angiogenic property and promote wound healing by promoting proliferation and migration of fibroblasts and keratinocytes cells.
- Conjugation of rGO with CTGF using EDC-NHS chemistry is a novel approach to accelerate the wound healing process.
- In the current work, we have developed a rGO/CTGF incorporated GelMA hydrogel dressing to improve wound healing by increasing proliferation and migration of cells as well as promoting formation of new blood vessels for increased supply of nutrients, oxygen and growth factors to wound area

Introduction

- **Background**
- Approximately, 170 million people in the world has effected by diabetes [1].
- The foot ulcers in diabetic patients is a leading cause of amputation. [4]
- A delayed healing or non-healing of wound in a person with DFUs is due to the reduction of growth factor response and decreased cells, which lead to reduced peripheral blood flow and decreased angiogenesis.
- **Solution**
- Recently, CTGF and reduced Graphene oxide (GO) have attracted great interest in biomedical applications due to its potential to enhance angiogenesis in wound healing applications. [2]
- GelMA hydrogel contain excellent porous structure and hydrophilic properties to mimic the properties of natural extra cellular matrix (ECM).
- Hydrogels are biocompatible, non- antigenic, durable, permeable to water vapour, and maintain its physical structure even after excessive water absorption. [5-7]
- **Aim of this study**
- In this study, rGO/CTGF incorporated GelMA hydrogel were synthesized using a simple UV crosslinking method.
- The hydrogels are then characterized (SEM, FTIR, TGF, DMA) to confirm the morphology of the material.
- The cytotoxicity of the hydrogel (Live/Dead, MTT assay) using hacat keratinocyte cells confirmed the biocompatibility of the material.
- Finally, the wound healing scratch assay shows the ability of the material to promote faster healing of the wound.

Materials and Methods

Conjugation of rGO with CTGF was done following the standard EDC/NHS chemistry. Pre-polymer solution of GelMA hydrogel was prepared following the method of Fatima et al [3]. 0.04%, 0.06% and 0.08% rGO/CTGF was dispersed in the GelMA solution and placed on a glass slide and then exposed to UV light (320– 500 nm, 7.0 mWcm2) for 10 seconds.

Results and Discussions

- The images of SEM shows highly porous structure of hydrogels both in top and cross-sectional view as shown in fig. 1A and 1B. The Transmission Electron Microscopy (TEM) mages of graphene oxide nanoparticles confirms the amorphous structure (fig. 1C and 1D).
- XRD spectra showed successful incorporation of rGO/CTGF nanoparticles in GelMA hydrogels.
- The Live/Dead assay shows the cell viability up to 0.06% concentration of rGO/CTGF. No significant cytotoxic effect was observed up to 0.06% concentration of rGO/CTGF.
- At 0.06% concentration of rGO/CTGF the wound was induced to closure comparatively to control, blank GelMA and 0.04% and 0.08% concentration of rGO/CTGF.
- GelMA hydrogel containing 0.06% rGO/CTGF nanoparticles produced large number of blood vessels with highly branched capillary network in chick embryo model compared to blank GelMA hydrogel treatment only.

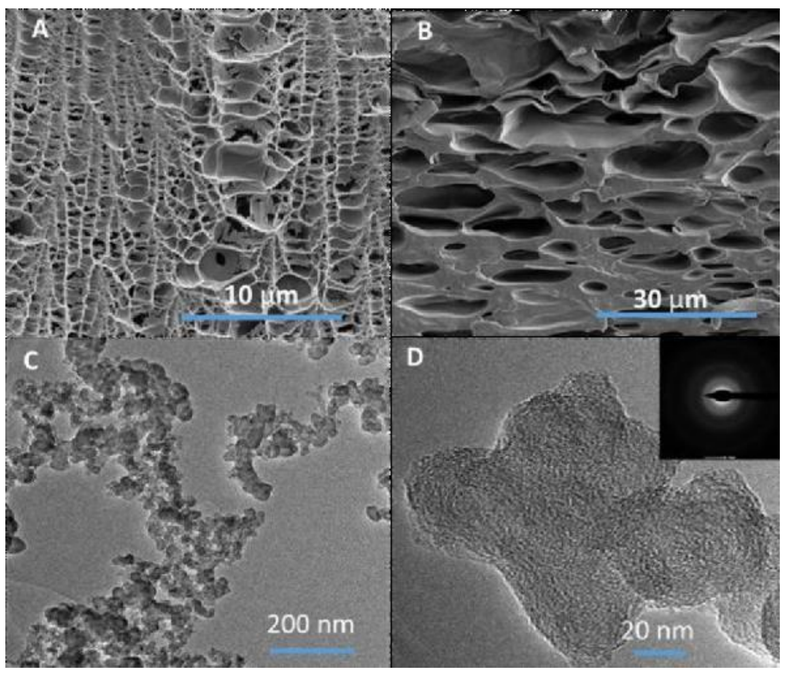

Figure 1: Fig. 1: Fig (1): SEM micrograph (A) Top view of GelMA hydrogel (B) cross-sectional view of GelMA hydrogel. (C) rGO nanoparticles (D) TEM image of amorphous GO nanoparticles.

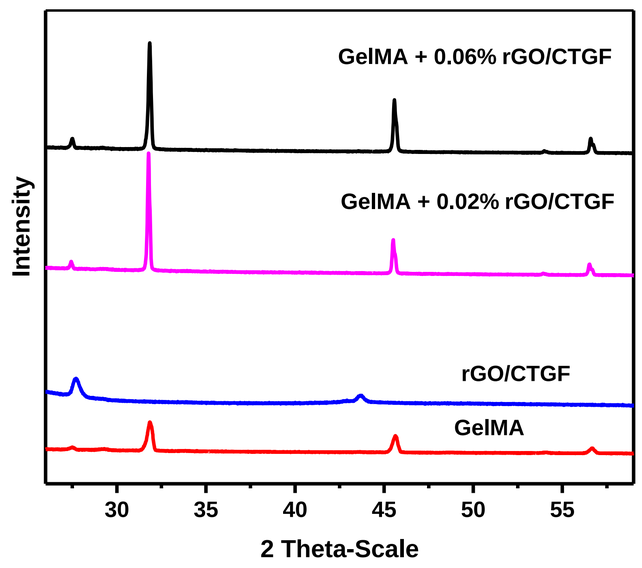

Figure 2: XRD of 0.06% rGO/CTGF loaded GelMA hydrogel, 0.02% rGO/CTGF loaded GelMA hydrogel, rGO and GelMA hydrogel respectively

Conclusions

- This rGO/CTGF/GelMA hydrogel exhibited remarkable wound healing potential displaying improved keratinocytes proliferation and migration
- GelMA hydrogel containing 0.06% rGO/CTGF nanoparticles produced large number of blood vessels with highly branched capillary network in chick embryo model compared to blank GelMA hydrogel treatment only.
- We strongly believe that our study on rGO/CTGF/GelMA hydrogel will put forward the insight for the advancement of angiogenic treatment strategies for several diseases where angiogenesis plays a significant role.

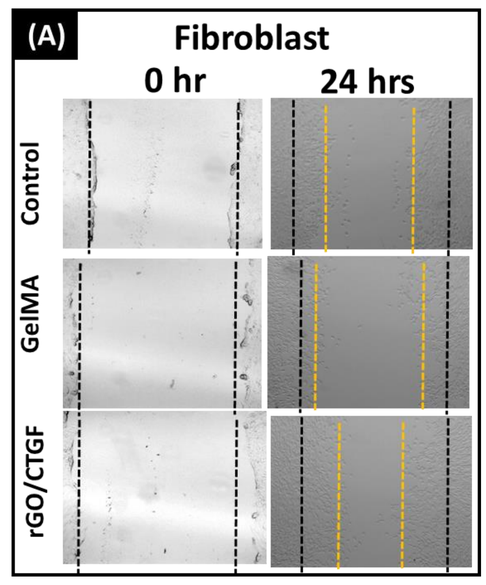

Figure 3: (A) Time dependant wound healing scratch assay of hacat keratinocyte cells from 0 hour to 24 hours for control, GelMA hydrogel, 0.062% concentration of GO loaded GelMA hydrogel respectively.

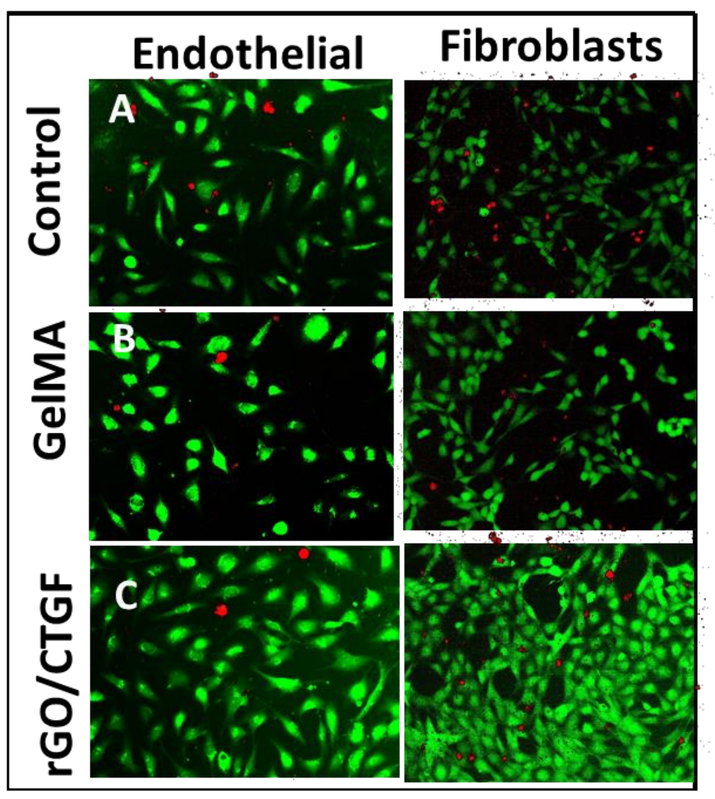

Figure 4 : Live/Dead cell assay of Control, Blank GelMA hydrogel and 0.06% rGO/CTGF loaded GelMA hydrogel respectively.

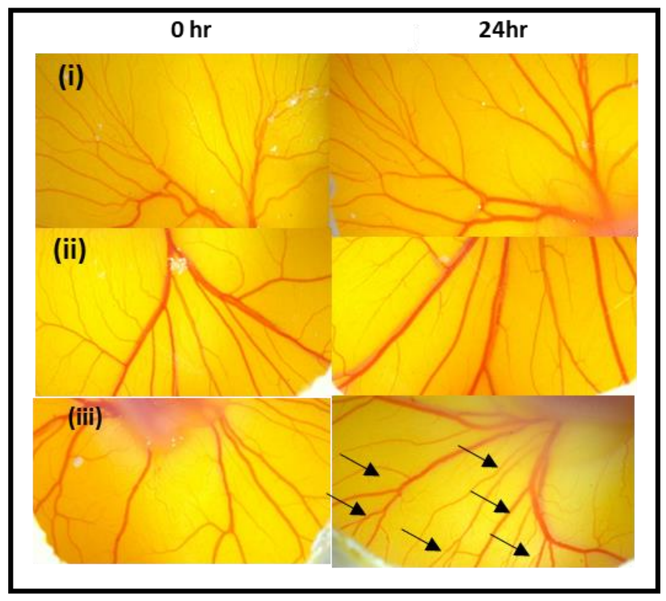

Figure 5 Blood vessel formation in chicken chorioallantoic membrane (CAM) assay after treatment of (i) pure GelMA (ii) GelMA with 0.06% rGO (iii) GelMA with 2% rGO.

Acknowledgements

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