Spectroscopic and Mechanical Studies of PEGDM and nHA-PEGDM Hydrogels

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***Abstract*—Hydroxyapatite is a key inorganic constituent in bone. Nano-hydroxyapatite was evaluated for its suitability as a Raman spectral marker for non-invasive osteogenesis monitoring using nHA-PEGDM hydrogels. Mechanical characterisation of PEGDM and nHA-PEGDM hydrogels assessed plasticity to gain insight into its performance as an implantable device.**

***Keywords*—Spectroscopy, hydrogel, hydroxyapatite, bone-on-chip, osteogenesis monitoring**

I. Introduction

Bone damage is a significant health concern in Europe, affecting over 100 million individuals, with osteoporotic bone damage alone costing €37.5 billion [1]. Traditional autologous bone grafting limitations have spurred interest in engineered bone implants for regenerative medicine. However, these implants have failure rates of up to 62% [2], emphasising the pressing need to enhance bone quality and decrease healing times.

Conventional assessment of implant success relies on invasive and time-consuming histology, typically performed 4-6 weeks post-implantation [3]. In contrast, a non-invasive approach focusing on mineral deposition could provide early detection of implant failure and scaffold optimisation before implantation.

Therefore, the development of a hydrogel-based bone-on-chip (BOC) device, combined with non-ionising optical spectroscopy such as Raman spectroscopy, offers a non-invasive means of longitudinally monitoring bone growth in implants (Figure 1). This not only enhances understanding of bone formation on hydrogel scaffolds but also allows evaluation of hydrogel scaffold quality.

Hydroxyapatite is a naturally occurring inorganic constituent of bone, and is expected to be present in increasing quantities on hydrogel substrates as bone growth occurs [5]. This enables it to serve as an ideal spectral marker for longitudinal Raman spectroscopic evaluation of osteogenesis on a BOC device. It has also been found to strengthen the mechanical properties of substances, which was evaluated herewith.

II. Materials and Methods

Due to its osteogenesis-promoting properties, poly(ethyleneglycol) dimethacrylate (PEGDM) hydrogels at concentrations of 10%, 15%, 20%, and 30% were formulated in phosphate buffered saline (PBS) [4]. These hydrogels were synthesised by curing under a UV light source at 400 nm for 15 minutes, facilitated by the photoinitiator, Irgacure 2959. The curing process was optimised with respect to Irgacure concentration and UV intensity to ensure the formation of stable hydrogels. Cryo-Scanning Electron Microscopy (Cryo-SEM) images were acquired using a Zeiss Ultra Plus SEM to visualise the hydrogel morphology [5].

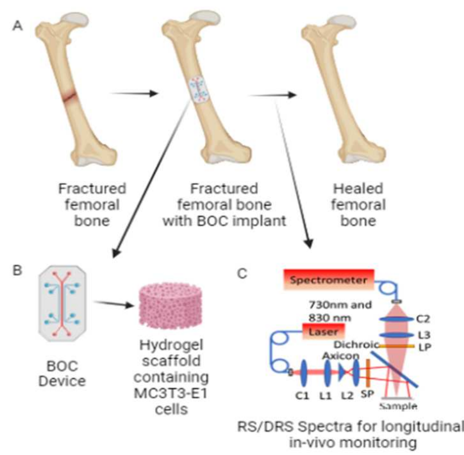

Figure 1: (A) Schematic representation of fracture healing with the aid of a BOC device. (B) BOC device containing osteoblast cells in a hydrogel scaffold. (C) In-vitro longitudinal data acquisition for osteogenic progression monitoring using optical spectroscopy.

Raman spectra were acquired at wavelengths of 730 nm, 785 nm, and 830 nm to encompass the full range of wavenumbers. Mechanical properties of PEGDM hydrogels were assessed using an Instron 5565 mechanical testing system, and Young's Modulus values were computed from acquired data.

The feasibility of Raman spectroscopy for osteogenesis monitoring was assessed by detecting hydroxyapatite (HA). Nano-hydroxyapatite at concentrations of 5%, 6%, and 7% was added to PEGDM hydrogels ranging from 10% to 30%. Spectra were acquired at 730 nm and 830 nm, and mechanical characterisation was conducted as described above.

III. Results and Discussion

Consistent with existing literature, it was noted that the pore size of the hydrogel networks decreased as the PEGDM concentration increased [6].

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Figures 2A-C illustrates Raman spectra obtained at wavelengths of 730 nm, 785 nm, and 830 nm. Higher concentrations of PEGDM were found to correlate with increased levels of polymerisation, leading to increased spectral intensities of bonds associated with the PEGDM polymer (C-C, C-O, C-H), while bonds undergoing photopolymerisation (C=C, C=O) showed decreased intensities. Additionally, a decrease in the intensity of the OH peak at 730 nm (Figure 2C) was noted with rising polymer concentration, suggesting a greater degree of crosslinking and polymer density [7,8].

Distinct Raman spectra were observed for pure PEGDM and Irgacure 2959 powders and solutions compared to cured PEGDM hydrogels, reflecting the processes of polymerisation and the presence of water in the solution phase.

It was noted that PEGDM hydrogels increased in rigidity and became brittle with higher polymer concentration, as evidenced by increasing Young's Modulus values (Figure 2F).

In nHA-PEGDM hydrogels, characteristic spectral peaks associated with HA were observed at 961 cm^{-1} and 991 cm^{-1} (ν_1 $PO_4{}^{3-}$) and at 1185 cm^{-1} (ν_3 $PO_4{}^{3-}$) (Figure 2D-E), which corresponds to commercial HA [5]. These peak intensities were observed to increase with increased nHA incorporation into the hydrogel.

Moreover, the Young's Modulus of nHA-PEGDM hydrogels increased with higher nHA content, compared to a control hydrogel with no nHA added (Figure 2F). This increasing brittleness during bone growth makes mechanical testing another valuable means to assess osteogenesis. However, as the HA content approached 7%, the Young's Modulus was lower than both 5% and 6% nHA-PEGDM samples and the PEGDM control. This discrepancy suggests the inability of nHA to remain in suspension during the curing process, rather than the plasticity of the material at higher nHA concentrations.

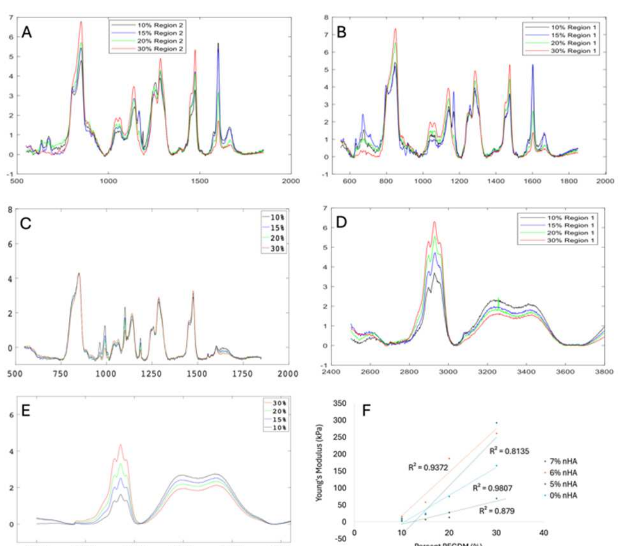

Figure 2: (A-C) Raman spectra of 10%-30% PEGDM hydrogels acquired at (A) 785 nm, (B) 830 nm and (C) 730 nm. (D-E) Raman spectra of 6% nHA-PEGDM hydrogels acquired at (D) 830 nm and (E) 730 nm. (F) Young's Modulus versus PEGDM concentration at various nHA concentrations.

IV. Conclusion

Variations in PEGDM, nHA, and nHA-PEGDM concentrations can be readily identified through morphological (cryo-SEM), spectroscopic (Raman spectroscopy), and mechanical characterisation (compression testing) techniques. The detection of nHA using Raman spectroscopy makes it an excellent candidate as a spectral marker for monitoring osteogenesis in hydrogel samples. The fabrication of microfluidic devices for further investigation is identified as next steps in this research study.

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