Luminescent 3D-Printed Bioactive Glass Scaffolds with Persistent Luminescent Particles for Bone Bioengineering

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Introduction

Complex and critical-size bone defects require innovative devices presenting osteoproperties: from osteocompatibility to osteogenesis. Due to its ability to release ions in solution and trigger signaling pathways leading to "osteocompetent responses", bioactive glass (BAG) appears as a pertinent solution to face the needs in bone engineering[1]. However, its limited drug delivery capacity remains a major limitation. To overcome this, we developed a hybrid platform combining 13-93B20 BAG scaffolds with Persistent Luminescent Microparticles (PeL-MPs), enabling photorelease of drugs in a spatiotemporal manner and potential low-light therapy[2,3]. A major goal was to assess the biocompatibility and stability of PeL-MPs throughout the bone healing process. Porous scaffolds of 13-93B20 BAG containing turquoise, blue, green, or red PeL-MPs were fabricated by extrusion-based 3D printing. The effect of these PeL-MPs on the scaffolds' properties and on Adipose Stem Cells (ASCs) behavior under subsequent excitation and emission was investigated. Bone regeneration was studied after scaffolds implantation into bilateral critical calvarial defects.

Material and Method

Scaffolds preparation

- **Glass 13-93B20**

Composition (mol%)						
Na_2O	K_2O	MgO	CaO	P_2O_5	SiO_2	B_2O_3
6.0	7.9	7.7	22.1	1.7	43.7	10.9

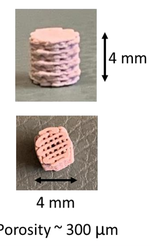
- **Persistent luminescent (PeL) MPs**
  - **Turquoise** ($Sr_4Al_{14}O_{25}$:Eu;Dy)
  - **Blue** ($Sr_2MgSi_2O_7$:Eu,Dy)
  - **Green** ($SrAl_2O_4$:Eu,Dy)
  - **Red** (Ca,Sr)S:Eu)

- **Scaffolds processing by robocasting**

13-93B20 (< 38 µm) — 90% (wgt %)
Pel particles (50-100 µm) — 10% (wgt %)

Scaffolds obtained by 3D-Printing

Sintering at 635 °C 1h



Porosity ~ 300 µm

Scaffolds characterization



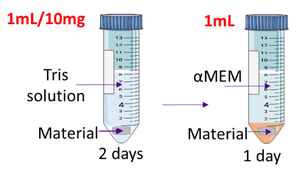
- **Dispersion of the persistent luminescent MPs by SEM**
- **Luminescence observations of the scaffolds after charging with UV lamp and confocal microscope laser**
- **Luminescence of the scaffolds after immersion in culture medium**



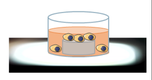
Scaffolds immersed up to 14 days in culture medium

Biological response

- **Pre-incubation step**



- **Cell culture step**



White light

Conditions:
- ❑ 16 000 Adipose Stem Cells (ASCs) seeded in each well
- ❑ 1mL of medium, αMEM 5% Human Serum, 1% Peni/Strepto
- ❑ Scaffolds charged with white light 15min every hour

- ***In-vivo* study**

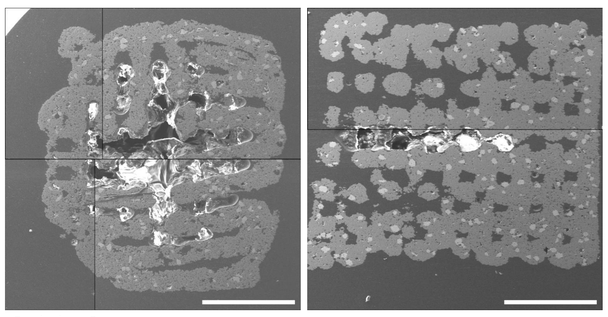


Implantation in a critical-size calvarial defect in rats for 3 months

Scaffolds characterization

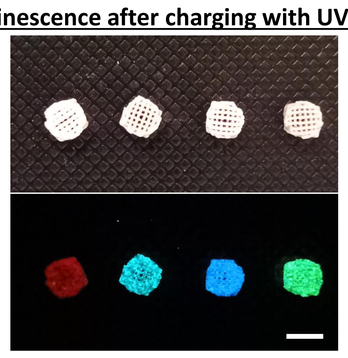
Dispersion of PeL MPs in the scaffolds and luminescence observations

Dispersion of PeL MPs in the scaffolds observed by SEM



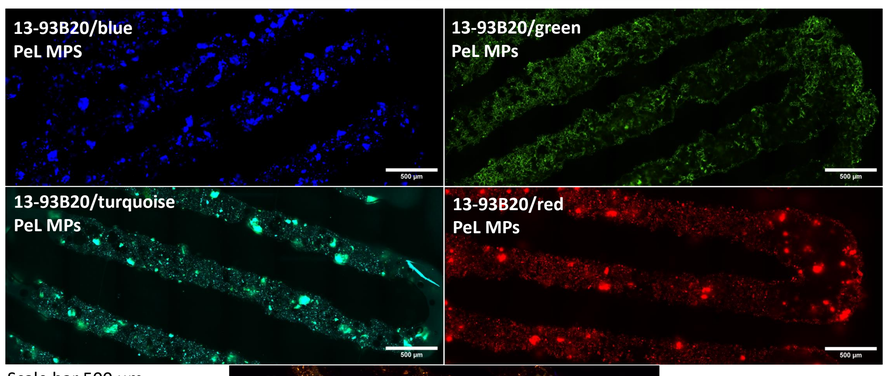
Scale bar 2 mm

Luminescence after charging with UV lamp

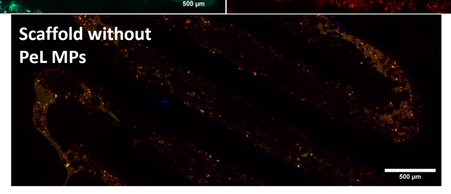


Scale bar 4 mm

Luminescence after charging with confocal microscope laser



Scale bar 500 µm

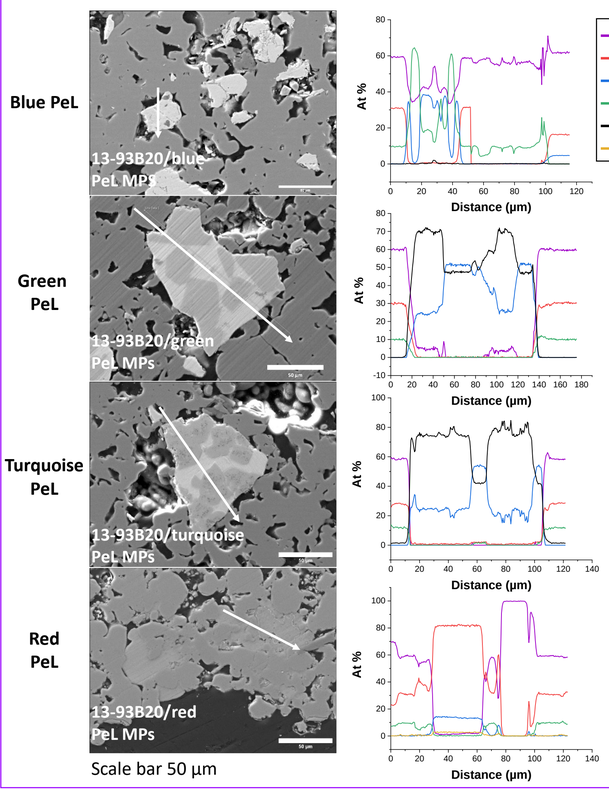


- **Homogeneous dispersion of PeL MPs in the scaffolds**
- **Luminescence properties of pel particles conserved after 3D-printing and sintering**

➢ **Synthesis technique allows a homogeneous dispersion of PeL MPs in the scaffolds while maintaining their luminescence properties**

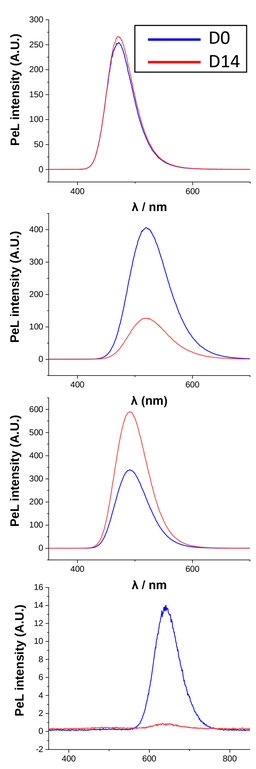
Immersion test and luminescence quantification

Atomic quantification after 14 days of immersion in culture medium



Scale bar 50 µm

Luminescence intensity

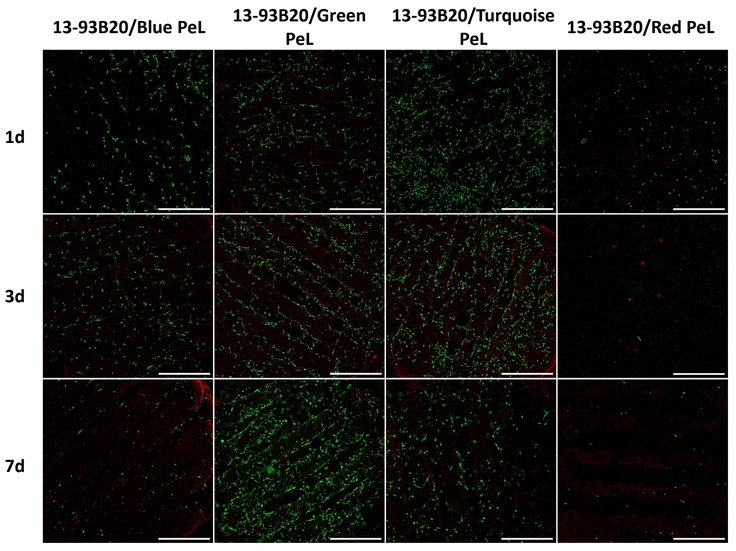


- ➢ **Luminescence not impacted by the immersion in culture medium**
- ➢ **Decrease in PeL post-immersion → probably due to particle dissolution and/or mineralization**
- ➢ **Luminescence higher after 2 weeks of immersion → probably due to an excess of luminescence inside the scaffolds after immersion**
- ➢ **Complete loss of luminescence post-immersion → dissolution of the PeL particles**

Cellular characterization

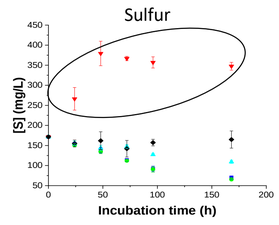
Toxicity of PeL MPs without charging them with white light

ASCs viability on the scaffolds by Live-Dead Staining



Scale bar 1000 µm

Elemental quantification by ICP-OES

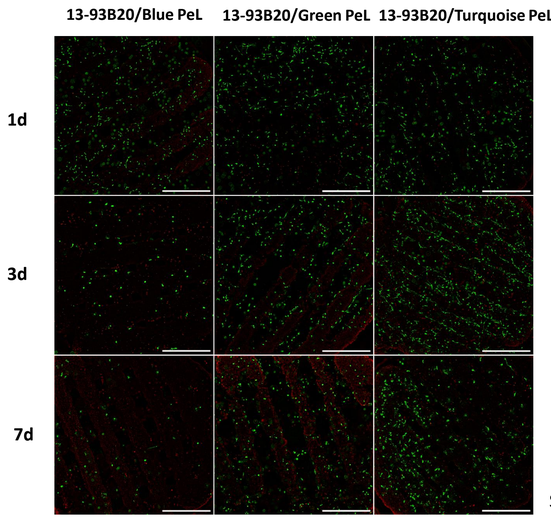


- **Cells alive on top of scaffolds with green and turquoise PeL MPs**
- **Amount of cells lower on scaffolds with blue PeL MPs**
- **Dead cells on top of scaffold with red PeL MPs → toxicity of red PeL MPs ?**

➢ **Red PeL MPs probably toxic due to a high and fast release of S and increased rate of glass dissolution**
➢ **Blue PeL MPs degrade in solution leading to an increased glass dissolution rate**

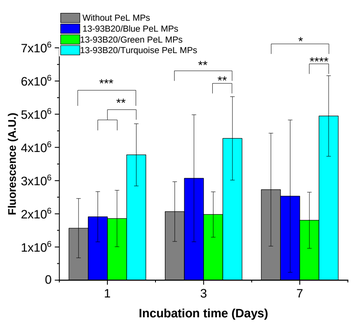
Effect of the luminescence on cell behavior

ASCs viability on the scaffolds by Live-Dead staining



Scale bar 1000 µm

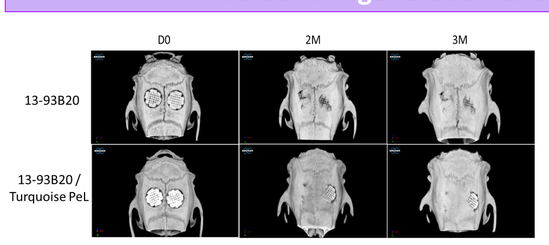
ASCs proliferation on the scaffolds by Cyquant analysis

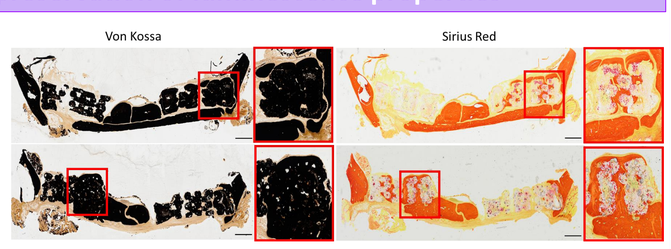


*p < 0.05
**p < 0.005
***p < 0.001
****p < 0.0001

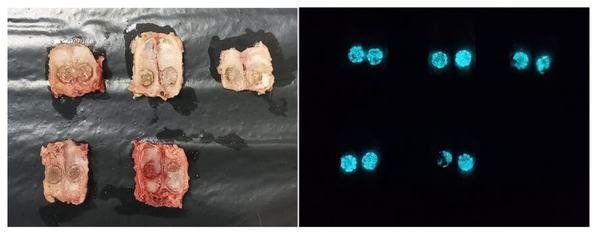
➢ **Turquoise PeL MPs seem to promote cell viability and cell proliferation compared to blue and green Pel MPs.**

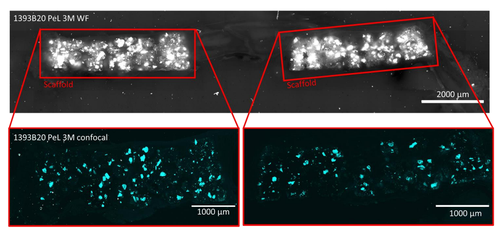
In-vivo bone regeneration and preservation of luminescence properties





➢ **Turquoise PeL MPs do not prevent bone regeneration in the defect through the scaffold**





➢ **Luminescence is preserved after three months of implantation**

Conclusion and Perspectives

This project aims to develop scaffolds for bone tissue engineering but also to allow drug release for a specific therapeutic response by adding persistent luminescent microparticles (PeL MPs) in 13-93B20 bioactive glass. Here, it is showed that the technique used to obtain the scaffolds allows the homogeneous dispersion of PeL MPs in glass scaffolds and the conservation of the luminescence. Turquoise PeL MPs seem to be the most promising for keeping the luminescence but also promoting cell behavior in the scaffolds and bone regeneration *in-vivo*. This work paves the way for light-assisted therapies, controlled release of photosensitive molecules, and bioimaging applications in hard tissue engineering.

Acknowledgements

The authors acknowledge the Biocenter Finland, Tampere Imaging Facility for the service and Tampere Microscopy Center facilities at Tampere University. The authors would like to thank Academy of Finland and Jane and Aatos Erkko foundation for providing financial support to this project.

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