Hyaluronic acid glycidyl methacrylate hydrogel gelation in turbid medium of biotissue

A.G. Savelyev[1,2], **A.V. Sochilina**[1,3], **R.A. Akasov**[1,2,3], **M.E. Nikolaeva**[4] , **N.A. Durandin**[5], **L. George**[5], **A. Efimov**[5], **V.A. Semchishen**[1], **A.N. Generalova**[1,3] **and E.V. Khaydukov**[1,2,3]

[1] Federal Scientific Research Centre "Crystallography and Photonics" of Russian Academy of Sciences, Moscow, Russia

[2] Sechenov First Moscow State Medical University, Moscow, Russia

[3] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia

[4] MIREA — Russian Technological University, Moscow, Russia

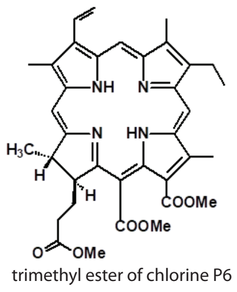
[5] Tampere University, Tampere, Finland

E-mail: a.g.savelyev@gmail.com

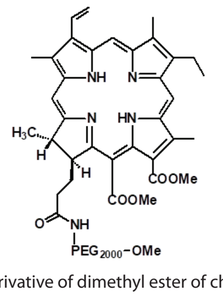
Introduction

Tissue engineering is an innovative field of medicine involved in regeneration and replacement of damaged or diseased tissues. In particular, the specially designed implant is integrated to the place of defect aiming to mimic physical and biological properties of the native tissue. Unfortunately, this approach remains not beneficial for repair of small defects in plastic surgery due to impossibility to place matrix in the depth of organism without surgery.
It motivated us to design injectable bioink on the base of hyaluronic acid glycidyl methacrylate and photoinitiators possessing absorption in the red region. For this reason we synthesized water-soluble 173-N-(methoxy-PEG2000)-131,151- chlorin p6 dimethyl ester and {4,4',4'',4'''-(29H,31H-phthalocyanine-1,8,15,22-tetrayl-134 κ4N29,N30,N31,N32)tetrakis[1- methylpyridiniumato(2-)]}zinc(4+) tetraiodide. Herein we have studied implementation of these photoinitiators *in vitro* and *in vivo* for crosslinking under irradiation at 660/675 nm wavelengths.

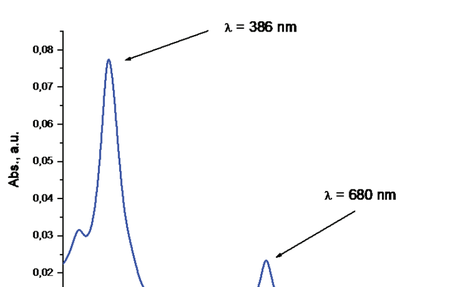
173-N-(methoxy-PEG2000)-131,151- chlorin p6 dimethyl ester



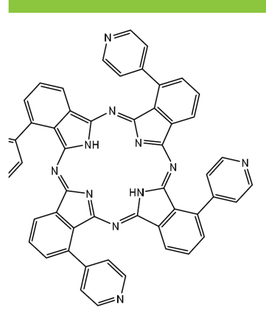
trimethyl ester of chlorine P6



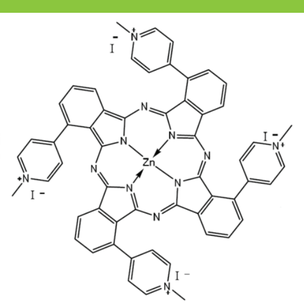
PEG – derivative of dimethyl ester of chlor



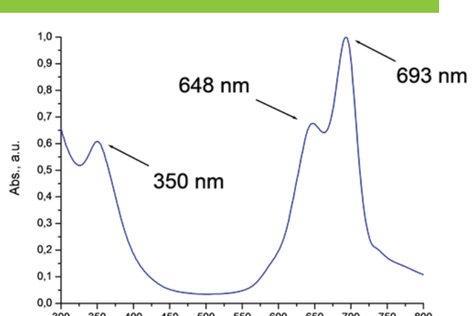
4,4',4'',4'''-(29H,31H-phthalocyanine-1,8,15,22-tetrayl-134 κ4N29,N30, N31,N32)tetrakis[1- methylpyridiniumato(2-)]}zinc(4+) tetraiodide



Phtalocyanine



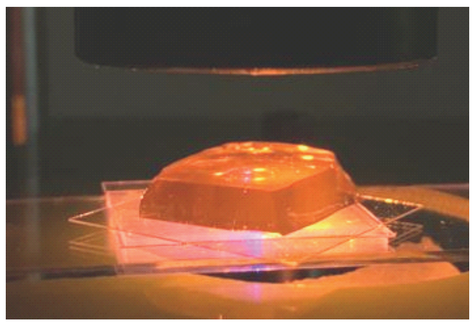
Derivative of phtalocyanine



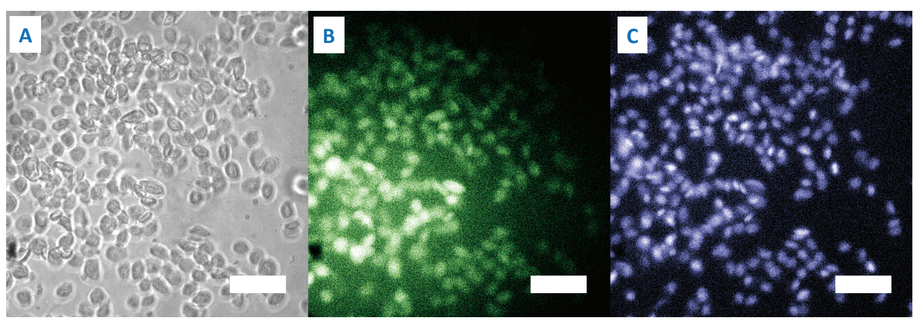
Preparation of photocurable compositions

The photocurable composition consists of monomer or polymer with double bonds and photoinitiator. Derivatives of trimethyl ester of chlorine P6 or phthalocyanine are tested as photoinitiators. In all water-soluble PCCs we used poly(ethylene glycol) diacrylate (PEGDA) or hyaluronic acid glycidyl methacrylate (HAGM) as base polymers disolved in water solution containing photoinitiator.

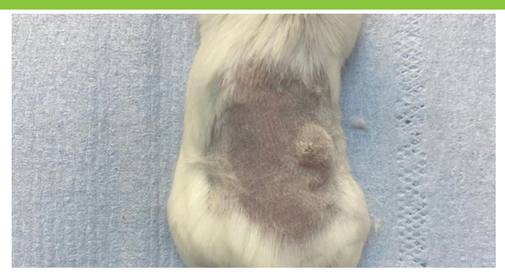
In vitro tests

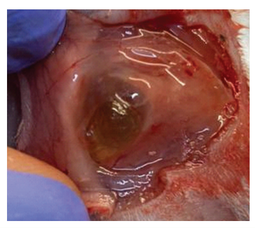


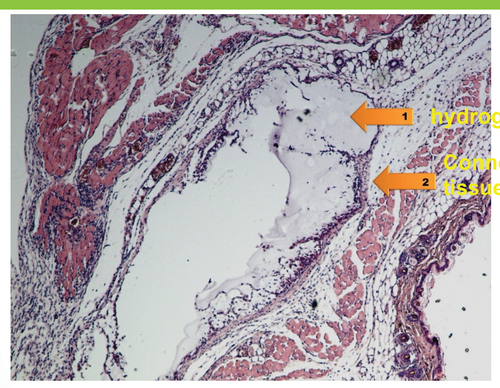
Photocrosslinking of cell-laden hyaluronic acid glycidyl methacrylate (HAGM) hydrogel under 5 mm agarose phantom mimicking living tissue has been demonstrated. Cell (HaCat) viability has been confirmed by confocal microscopy after 1 day of incubation in growth medium: A – optical microscopy, B – Calcein AM fluorescent imaging (alive cells, in green), C – Hoechst 33342 fluorescent imaging (cell nucleuses, in blue). Scale bar 50 μm.



In vivo tests

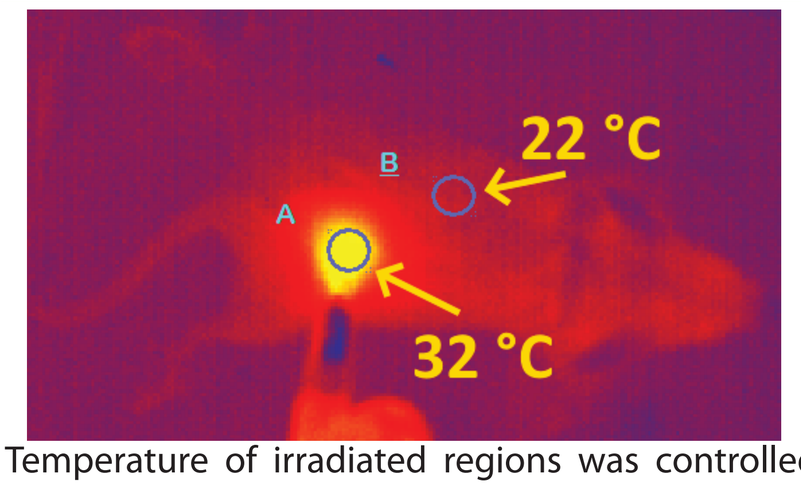

Hydrogel injection and irradiation at 675 nm





Tissue response:
- lack of pronounced inflammatory response
- lack of pronounced lymphocyte infiltration
- implant is covered by thin connective tissue capsule

Temperature control



Temperature of irradiated regions was controlled using a Gobi-384-GigE-7098 infrared camera (Leuven, Belgium).

Conclusions

Our concept demonstrates proof of principle of new minimally invasive technology for *in situ* tissue engineering construction formation in the depth of living organism. Relatively low concentration (200 μM) of photoinitiator in the combination with irradiation (660-675 nm) within biotissue transparency window will permit to produce hydrogel gelation without of cell destruction.
Hydrogel samples with both photoinitiators indicated high biocompatibility confirmed by lack of systemic or local inflammatory effects at the macro level. On the 30th day the thin granulation tissue was formed around the hydrogel sample.

Acknowledgements

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