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Fabrication of porous hydrogels containing hyaluronic acid by photoinduced crosslinking

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Abstract. Biocompatible hydrogels have gained much attention in tissue engineering, preferably as scaffolds providing the cell attachment and viability in the hydrogel bulk. This requires fabrication of the hydrogels with pores, the sizes of which are in the range of 100-300 μm, most optimal for cell growth. The composition of hydrogels or method of fabrication may affect the formation of porous structure. We prepared hydrogels via photoinduced crosslinking of hyaluronic acid modified with glycidyl methacrylate under irradiation at different wavelengths using two photoinitiators. The hydrogel structure was varied by blending hyaluronic acid derivative with other modified polymers of natural origin (gelatin and pullulan) with grafted vinyl moieties or using filler (sucrose). The most optimal pore sizes for cell growth were obtained for hydrogels derived from modified hyaluronic acid, with the addition of sucrose or processed with the single freeze-thaw cycle. The produced hydrogels demonstrated lack of cytotoxicity with HaCaT cells incorporated inside gel bulk.

Keywords: pores, scaffolds, photoinduced crosslinking, biopolymers, bioink

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Формирование пористых гидрогелей, содержащих гиалуроновую кислоту, методом фотоиндуцируемой сшивки

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Аннотация. Биосовместимые пористые гидрогели привлекают большое внимание в тканевой инженерии, преимущественно в качестве скаффолдов, обеспечивающих прикрепление клеток и их жизнеспособность в объеме гидрогеля. В данной работе была исследована структура гидрогелей, полученных разными способами на основе модифицированных полимеров природного происхождения методом фотоиндуцированной сшивки. Наиболее оптимальные размеры пор для роста клеток были у гидрогелей, сформированных из модифицированной гиалуроновой кислоты с добавлением сахарозы или обработанных с помощью однократного цикла замораживания-оттаивания. Полученные гидрогели продемонстрировали отсутствие цитотоксичности на примере культуры клеток HaCaT, включенным в объем геля.

Ключевые слова: поры, скаффолды, фотоиндуцируемая сшивка, биополимеры, биочернила

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Introduction

Biocompatible hydrogel scaffolds have recently gained increasing interest in tissue engineering, while manufacturing suitable scaffolds for cell culture and tissue regeneration remains an intensive area of development [1]. Cell attachment and viability are strongly correlated with pore sizes. The average size of animal cells is ~10–30 μm, therefore the most optimal sizes of hydrogel pores for their growth and development are within 100 to 300 μm [2]. Porous matrix can be produced by various methods such as fiber bonding, salt leaching, foaming and 3D printing. Moreover, the various components of hydrogel can exert influence on the formation of pore sizes during gelation [3]. In our study, the emphasis was made on the evaluation of the factors which can tune pore sizes.

Materials and methods

Photocompositions (inks). All inks for the formation of hydrogels were made on the basis of modified polymers of natural origin – hyaluronic acid (mHA), pullulan (mPul) and gelatin (mGel) – prepared by using the method of grafting moieties with double bonds through

polymer-analogous reaction with glycidyl methacrylate [4]. Sucrose (20%) was added to the composition of some inks to study the effect on the pore formation. In order to activate the process of crosslinking by irradiation upon wavelengths of 450 and 660 nm, two photoinitiators of the type II were used: flavin mononucleotide (FMN) and pyridine-substituted phthalocyanine (Pht). Co-initiators chosen from compounds with amino groups (for FMN) and with thiol groups (for Pht) provide the efficiency of photoinitiators.

Photo crosslinking. In order to form hydrogels of the equal volume, silicone molds opened on both sides (height 1 mm, diameter 6 mm) were used. The ink was placed in a mold clamped between two slides and exposed with laser radiation at wavelength of 450 nm (600 MW power) for 15 min for each side of the mold if FMN was contained [5], or 660 nm (2500 MW power) for 15 min on one side of the mold if Pht was contained. Different power and time were used to ensure efficient crosslinking and formation of stable hydrogels.

Pore size measurement. The gel pore images were acquired using a scanning electron microscope (Phenom ProX, Thermo Fisher Scientific) after hydrogel shock freezing in liquid nitrogen and lyophilic drying.

***In vitro* study.** The human keratinocytes (HaCaT cell line) were entrapped into the ink during fabrication, and their growth within the gel bulk was demonstrated with fluorescent dye Calcein-AM using fluorescent microscopy.

Results and Discussion

The pore sizes of formed gels varied approximately from 5 to 100 μm depending on the constituents. The pore sizes of hydrogels based on only mHA (Fig. 1,*a*) containing either FMN or Pht almost did not differ and were in the range of 30–50 μm, at the same time Pht gels possessed slightly larger pores with ragged edges. The smallest pores (2–20 μm) were evaluated in the hydrogels based on mGel and mPul, as well as in their mixtures with mHA (Fig. 1, *b*, *c*). Insignificant changes in pore size during the treatment of mixtures of mHA with mGel and mPul with the enzyme hyaluronidase, as well as their more prominent enzymatic degradation compared to hydrogels based on pure mHA, suggested that mGel and mPul make the main contribution to the formation of the structure. Limited mHA crosslinking was likely due to the high level of affinity for water compared to other biopolymers.

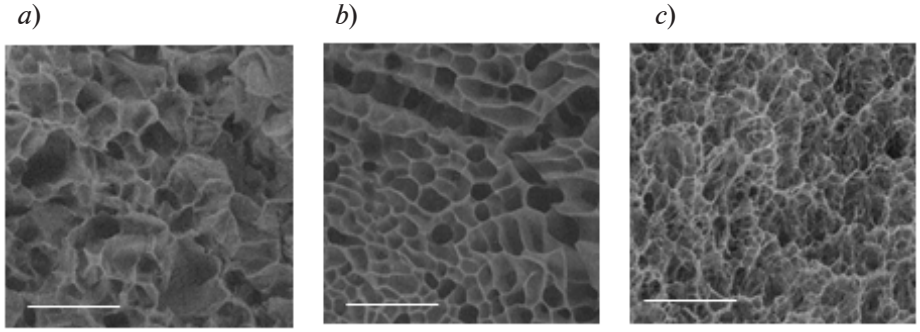

Fig. 1. SEM images of gels containing: mHA 20% (*a*); mHA 10% and mGel 10% (*b*); mHA 10% and mPul 10% (*c*). Scale bar 50 μm

It was found that scaffolds contained from mHA 20% after treatment with hyaluronidase resulted to substantial increase in pore sizes (from 100 μm) (Fig. 2,*a*). Moreover it was discovered that utilization of sucrose, which can be removed after irradiation by swelling in aqueous solutions, led to the formation of larger pores (50–100 μm) in hydrogels (Fig. 2,*b*). The same effect can be achieved by using the freezing-thawing technique for polymer compositions in the range of temperatures from −30 to −5 °C, when even more significant increase in pore size (100–150 μm) was observed (Fig. 2,*c*).

The in vitro study of HaCaT cells embedded in hydrogel bulk demonstrated fluorescence of Calcein-AM (Fig. 3), indicating living cells in both FMN and Pht gels (mHA 20%) during 3 days of experiment, which confirms good cell viability in such environment.

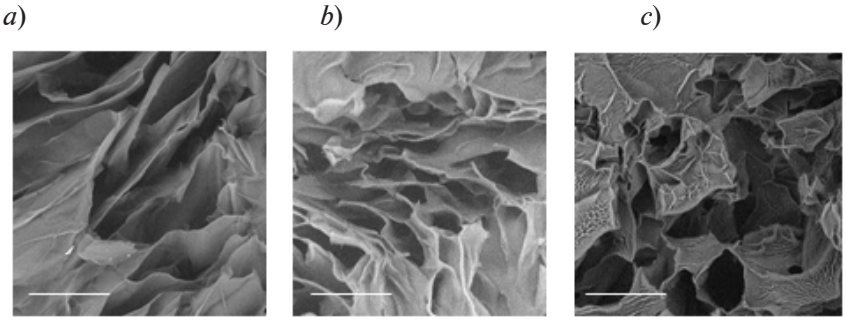

Fig. 2. SEM images of gels containing: mHA 20% after treatment with hyaluronidase (scale bar 50 μm) (*a*); mHA 20% and sucrose 20% (scale bar 50 μm) (*b*); mHA 20%, after freeze-thawing cycle treatment (scale bar 100 μm) (*c*)

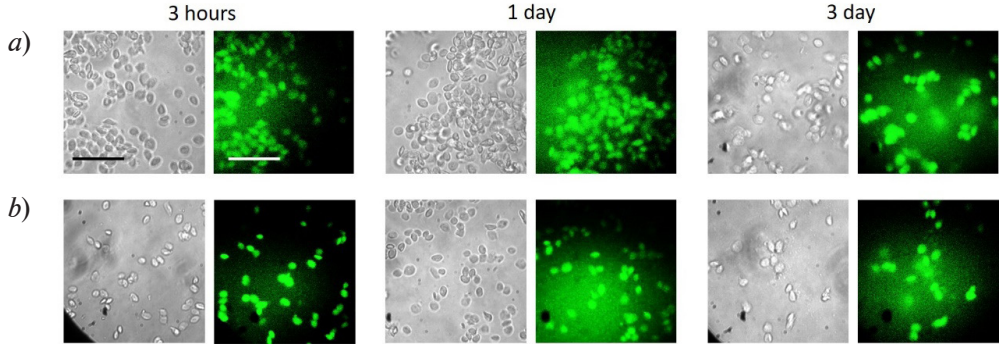

Fig. 3. Fluorescence of HaCaT cells inside of hydrogels containing: FMN (*a*); Pht (*b*). Scale bar 100 μm

Conclusion

Hence, the most auspicious hydrogel scaffolds are produced from mHA with the addition of sucrose or treated with a single freeze-thaw cycle, since they have the most optimal pore size for cell growth. Furthermore, the developed compositions can be used as bioinks with incorporated cells.

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