# Modified Hyaluronic Acid Scaffolds for Tissue Regeneration

**R. Kutraitė, J. Gaidemauskaitė, L. Ščiupakovaitė, D. Narauskaitė, A. Baltrukevičiūtė, O. Baniukaitienė***

*Department of Polymer Chemistry and Technology, Kaunas University of Technology, Lithuania*

*E-mail: odeta.baniukaitiene@ktu.lt*

**Introduction.** Nowadays various grafting materials are used for soft and hard tissue regeneration. Natural polymers are gaining considerable attention due to their potential application for tissue engineering [1-3]. Significant attention is focused on hyaluronic acid (HA). It is a linear polysaccharide composed of repeating disaccharide units of glucuronic acid and N-acetyl-D-glucosamine linked by alternating glycosidic bonds β - (1, 4) and β- (1, 3) (Fig. 1).

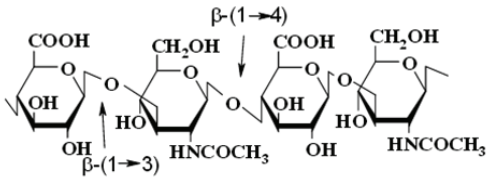


**Fig. 1.** Chemical structure of hyaluronic acid

In most cases, HA is used as viscous solutions or hydrogels obtained by chemical modification of HA. Chemical modification of HA has been used to obtain longer resistance time *in vivo* [4, 5]. However, HA gel or solution has no morphology, which is particularly important for cell adhesion, proliferation, differentiation, vascularisation, and for prolonged therapeutic action.

Modification of polymeric matrix with inorganic particles once changing its properties is a new direction in tissue engineering. As bioactive materials can be used: silicon dioxide, hydroxyapatite, silver particles and others [6].

The aim of this work was to prepare 3D HA scaffolds coated with $SiO_2$ particles.

**Materials and methods.** HA gel was prepared by crosslinking polymer in an alkaline solution by 1,4-butanediol diglycidyl ether. Further polymer modification was followed by tetraethoxysilane hydrolysis and condensation reactions [2]. After separation from solution, the gel was dried using freeze-drying technique (Christ ALPHA 2-4 LSC freeze dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Germany). For the morphological visualization of the scaffolds a high resolution field emission scanning electron microscope (SEM) Quanta 200 FEG (FEI Company, Netherlands) was used. Chemical composition of the scaffolds was analysed using Ouantax EDS system (Bruker AXS Microanalysis GmbH, Germany). Structural stability of

the prepared scaffolds was determined by immersing the scaffolds in phosphate buffer solution (PBS, pH = 7.4) at 37 ºC for up to 2 months.

**Results**. The modified HA scaffolds were prepared by crosslinking polymer in an alkaline solution by 1,4-butanediol diglycidyl ether and coating with $SiO_2$ microparticles. A highly porous structure of the scaffolds was obtained by freeze-drying. As demonstrated by the SEM photograph (Fig. 1) the scaffolds were composed of different pore sizes. The largest pores were of 220 μm.

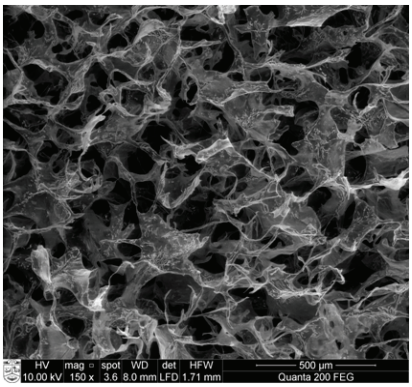


**Fig. 1.** SEM image of highly porous HA scaffold

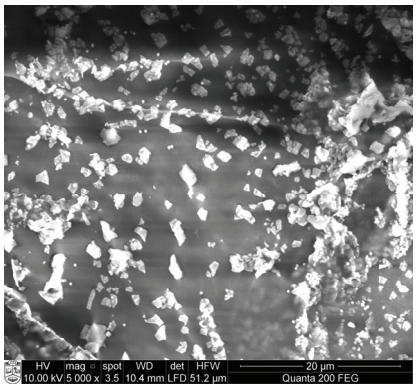


**Fig. 2.** SEM micrograph of the surface of HA scaffold coated with $SiO_2$

Small aggregates appeared on the surface of the scaffolds after tetraethoxysilane hydrolysis and condensation reactions (Fig. 2). Following on, the coated HA scaffolds were analysed for elemental composition. The EDS spectra (Fig. 3) of the scaffolds showed the presence of carbon (C), oxygen (O), nitrogen (N) as the main elements of HA. Furthermore, in the spectra there was a strong silicon atom signal revealing $SiO_2$ presence on the polymer surface. In the spectra, there was also peak which belonged to sodium and this was due to the used form of HA (sodium salt of HA).

In addition, the presence of $SiO_2$ particles in the originally prepared HA scaffolds clearly improved their structural stability. The results showed that crosslinked HA scaffolds were stable up to 1 month, while scaffolds further coated with $SiO_2$ particles were stable up to 2 months (Fig. 4).

**Conclusions.** Hyaluronic acid scaffolds were successfully coated with silicon dioxide particles by tetraethoxysilane hydrolysis and condensation reactions. The presence of silicon dioxide particles in the prepared HA scaffolds improved their structural stability. The modified scaffolds were highly porous and stable up to 2 months.

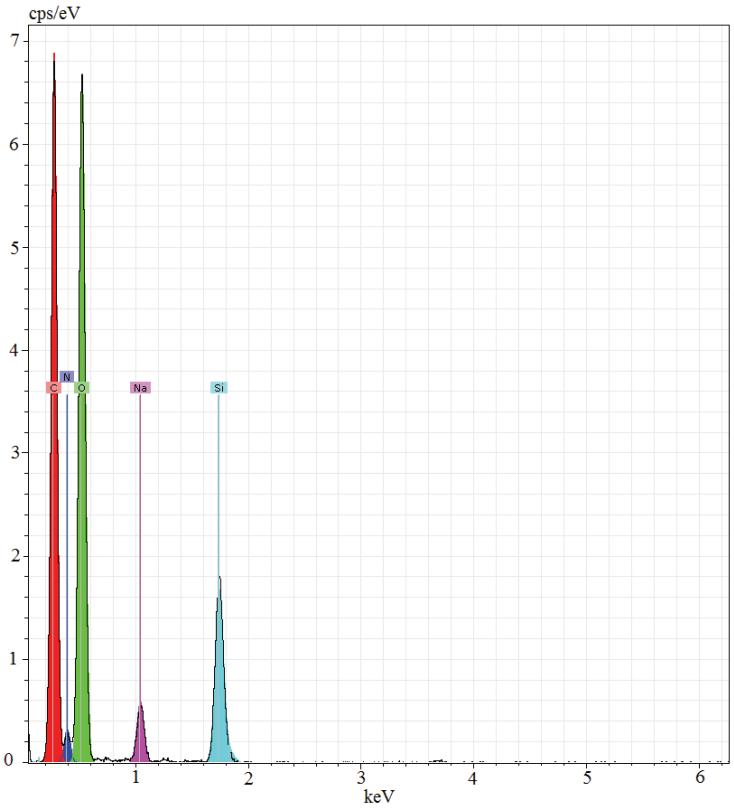


**Fig. 3.** Elemental composition of modified HA scaffolds

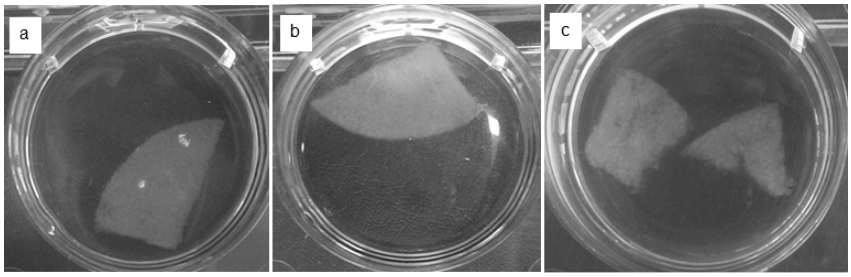


**Fig. 4.** Structural stability and degradation *in vitro* of coated HA scaffolds after 1 day (a), 2 weeks (b) and 2 months (c) of immersion

## References

1. Dhandayuthapani B., Yoshida Y., Maekawa T., et al. Polymeric scaffolds in tissue engineering application: a review. International Journal of Polymer Science. 2011, vol. 2011, p. 1-19.

2. Petrauskaite O., Gomes P., Fernandes M.H., et., al. Biomimetic mineralization on a macroporous cellulose-based matrix for bone regeneration. BioMed Research International. 2013, vol. 2013, p. 1-9.

3. Petrauskaite O., Juodzbalys G., Viskelis P., et al. Control of a porous structure of cellulose-based tissue engineering scaffolds by means of lyophilisation. Cellulose Chemistry and Technology. 2016, vol. 50, p. 23-30.

4. Brown T. J., Laurent U. B. G., Fraser J. R. Turnover of hyaluronan in synovial joints: Elimination of labelled hyaluronan from the knee joint of the rabbit. Experimental Physiology. 1991, vol. 76, p. 125-134.

5. Sadick N., Karcher C., Palmisano L. Cosmetic dermatology of the aging face. Clinics in Dermatology. 2009, 27, S3-S12.

6. Jesus C.G., Xiongbiao C., Qiaoqin Y. Effect of Nanoparticle incorporation and surface coating on mechanical properties of bone scaffolds: a brief review. Journal of Functional Biomaterials. 2016, vol. 7, p. 1-11.

**Modified Hyaluronic Acid Scaffolds for Tissue Regeneration**

**R. Kutraitė, J. Gaidemauskaitė, L. Ščiupakovaitė, D. Narauskaitė, A. Baltrukevičiūtė, O. Baniukaitienė**

*Department of Polymer Chemistry and Technology, Kaunas University of Technology, Lithuania*

In this work modified hyaluronic acid scaffolds for tissue engineering applications were prepared. Silicon dioxide particles appeared on the surface of the scaffolds after tetraethoxysilane hydrolysis and condensation reactions. The results obtained in this work revealed that the morphology of the scaffolds is suitable for soft and/or hard tissue regeneration. The scaffolds comprised non-symmetrical interconnected pores. Furthermore, the scaffolds were stable up to two months.