OPEN ACCESS
Journal of Medical Materials and Technologies
Vol 1, No 2, Special Issue Proceedings of 4th Euro BioMAT, Page No. 30-31 (2017)
http://journal.medmat.online
med:mat
ISSN: 2366-9136

Patterning of Versatile Nanocomposite Biomaterials based on Bioinert PEG-Hydrogels and Bioactive Nanohydroxyapatite and Cellular Responses to the Biointerfaces

[1]*Marga Lensen, [1]Axel Löbus, [1]Zhenfang Zhang

[1]Technische Universität Berlin, Straße des 17. Juni 124, 10623 Berlin, Germany
* Lensen@Chem.TU-Berlin.de; Tel.: +49-30-314-29555

Abstract – Hydroxyapatite (HAp) is one of the most important bioceramics for medical and dental applications, as it possesses excellent biocompatibility and is osteoconductive. However, pure HAp is difficult to shape in the complex forms required for tissue engineering applications such as bone repair because of its hardness and brittleness. Therefore, we have developed novel nanocomposite hydrogels based on HAp and poly(ethylene glycol) (PEG) hydrogels. The gel properties (soft, hydrated and moldable) allow the fabrication of 2D-patterned substrates and 3D-structured scaffolds, which can be further biomineralized to exhibit increased bioactivity and functionality to support the growth of osteoblast cells.

Keywords – cell adhesion; hydrogels; hydroxy apatite (HAp); nanocomposites; poly(ethylene glycol), PEG

1. INTRODUCTION

Poly(ethylene glycol) (PEG) hydrogels are well known to be non-toxic, non-fouling, non-immunogenic and are therefore widely used in cell-biological research [1]. PEG-hydrogels can be synthesized by different crosslinking mechanisms, which lead to tunable chemical, physical and elastic properties. We have synthesized PEG-hydrogels from macromolecular building blocks, exhibiting different molecular weights and architecture, and which contain different crosslinking groups, i.e. acrylate or vinyl sulfone, or azide/alkyne functionalities.

PEG-building blocks with acrylate end-groups are very versatile, since they can be crosslinked by different chemistries. The most common way is by radical crosslinking; using a photoinitiator and UV-illumination. This method leads to effectively crosslinked polymeric networks that are not soluble in water, yet may imbibe great quantities of water, hence the name hydrogels.

Recently, we have discovered that acrylate-functionalized PEG-macromonomers may be crosslinked by virtue of amine-ene Michael-type addition reactions, using ammonia as the reactant [2], or using thiols in thiol-ene Michael-type addition chemistry. A similar chemical strategy can be applied for vinyl sulfone-equipped macromonomers.

Moreover, we have decorated PEG-building blocks with azide and terminal alkyne groups, which are mutually reactive ("click chemistry") and can form relatively well-defined and controlled polymeric networks.

While PEG-hydrogels effectively prevent interactions with proteins and cells, we have revealed that modifications on the surface properties, such as applying nano- or micro-sized topography, (bio)chemical surface patterning or changing the elastic properties, induce remarkable cell adhesion [3-5].

Hydroxyapatite (HAp) is one of the most important bioceramics for medical and dental applications, as it possesses excellent biocompatibility and is osteoconductive. However, pure HAp is difficult to shape in the complex forms required for tissue engineering applications such as bone repair because of its hardness and brittleness. Therefore, we have developed novel nanocomposite hydrogels based on HAp and poly(ethylene glycol) (PEG) hydrogels. The gel properties (soft, hydrated and moldable) allow the fabrication of 2D-patterned substrates and 3D-structured scaffolds, which can be further biomineralized to exhibit increased bioactivity and functionality to support the growth of osteoblast cells.

Aim of the study: Novel nanocomposite materials are developed to show enhanced, preferential and specific cell adhesion of bone-like cells (osteoblasts) over tissue-like cells (fibroblasts).

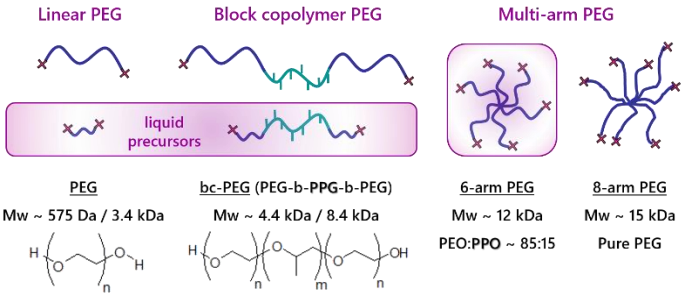
The aim of the study is further to control the location of specific cell adhesion by micro-patterning the biointerface; creating cell-adhesive areas (nanocomposites rich in hydroxyapatite) alongside bio-inert areas (pure PEG).

2. MATERIAL AND METHODS

Hydrogels

Depending on the requirement of the patterning method (*vide infra*), several PEG-based macromonomers can be selected from our library of multifunctional building blocks

[6], e.g. linear or star-shaped, liquid or solid, with a low or high molecular weight and with the desired functional groups for crosslinking the gels, see Scheme 1.



SCHEME 1.
PEG-MACROMONOMERS USED FOR MAKING PEG-HYDROGELS AND NANOCOMPOSITES.

Calcium Phosphate

In order to fabricate nanocomposite gels with hydroxyapatite (HAp), pre-formed nanoparticles can be (physically) mixed with PEG-prepolymers and the mixture subsequently crosslinked. Alternatively, the nanocomposites can be formed in situ. In that case, salt solutions are added to dispersions of the PEG-precursors in water, while stirring the reaction mixture. Figure 1 depicts a schematic sketch of the procedure.

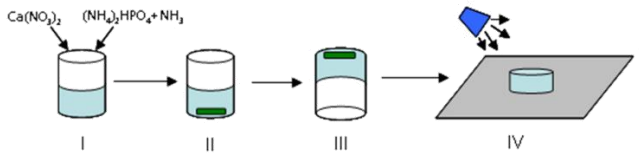


FIGURE 1
PREPARATION OF NANOCOMPOSITES OF PEG-HYDROGELS AND CALCIUM PHOSPHATE, E.G. HYDROXYAPATITE. I) ADDITION OF SALT SOLUTIONS, II) STIRRING, III) GELATION, IV) FINAL PHOTOINDUCED UV-CROSSLINKING.

3. RESULTS

Simple mixing of hydroxyapatite nanoparticles (HAp NPs) with the hydrogel precursors already yields novel nanocomposite materials with interesting properties. Nevertheless, even more finely dispersed HAp can be obtained when the PEG-precursors are mixed with salt solutions containing calcium and phosphate ions; in that case, nHAp is formed in situ. The nanocomposite precursor mixtures can be further crosslinked to create stable, new hydrogels with tunable mechanical and swelling properties. Interestingly, the sheer mixing of our 8-arm PEG-prepolymer (Mn≈15,000 Da) with salt solutions already resulted in spontaneous gelation, which is attributed to an amine Michael-type addition reaction taking place [2].

Films of the novel nanocomposites were characterized by several techniques, i.e. scanning electron microscopy (with EDX) and atomic force microscopy (both topography images and force mapping). It was observed that, notably in the case of the in situ formed nanocomposites, the calcium phosphate was distributed very homogeneously, and could be detected at the interface.

Cell culture studies with mouse fibroblasts (tissue cells) and pre-osteoblasts (bone cells) revealed that the presence of calcium phosphate at the interface hardly affected the affinity of fibroblasts to adhere, whereas the bone-like cells spread vividly on the nanocomposite material.

By virtue of their liquid state before crosslinking, the novel nanocomposite materials have been employed in our surface micro-patterning techniques that are based on soft lithography methods. In particular, our recently developed FIMIC-method (Fill-Molding in Capillaries, Figure 2) allows us to make topographically smooth, lateral patterns of bio-active (nHAp-PEG) verses bio-inert (pure PEG) materials.

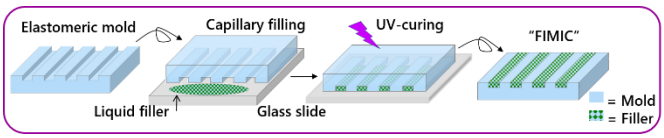


FIGURE 2
MICROPATTERNING OF NANOCOMPOSITE GELS BY THE FIMIC METHOD.

It has been observed that cells recognize the micro-patterns and adhere selectively.

4. CONCLUSIONS

We have been able to fabricate tailormade nanocomposite materials from PEG-hydrogels and calcium phosphate, especially hydroxyapatite. Several microscopic and spectroscopic investigations have corroborated the homogeneous distribution of the mineral phase within the gels.

While tissue cells (fibroblasts) hardly showed any response to the presence of hydroxyapatite at the biointerface, bone-like cells (pre-osteoblasts) responded more strongly, with significant adhesion and spreading. On micro-patterned samples this preference was even more clearly visible.

These preliminary results are very promising for tissue regeneration applications, notably for the development of bone repair strategies.

5. REFERENCES

[1] J. M. Harris. "Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications"; *Plenum Press*: New York, 1992.

[2] Zhang, Z.; Loebus, A.; Vicente, G. De; Ren, F.; Arafeh, M.; Ouyang, Z.; Lensen, M. C. *Chem. Mater.* 2014, 26, 3624–3630.

[3] Lensen, M. C.; Schulte, V.; Diez, M. "Cell Adhesion and Spreading on an Intrinsically Anti-Adhesive PEG Biomaterial." In *Biomaterials - Physics and Chemistry*; Pignatello, P. R., Ed.; InTech, 2011; pp. 397–414.

[4] V. A. Schulte, M. Díez, M. Möller, M. C. Lensen. *Biomacromolecules* 2009, 10, 2795.

[5] M. Diez, V. A. Schulte, F. Stefanoni, C. F. Natale, F. Mollica, C. M. Cesa, J. Chen, M. Möller, P. A. Netti, M. Ventre, M. C. Lensen. *Adv. Eng. Mater.* 2011, 13, 16.

[6] Susan Kelleher, Zhenfang Zhang, Axel Löbus, Christine Strehmel and Marga C. Lensen, *Biomaterials Science* 2014, 2 (3), 410 – 418.