RAFT POLYMERS AS ADHESION MOTIFS FOR BONE GLUE APPLICATIONS

Patrick Steinbauer[a,c], Andreas Rohatschek[b,c], Orestis Andriotis[b,c], Robert Liska[a,c], Philipp J. Thurner[b,c] and Stefan Baudis[a,c]

[a]E163 Institute of Applied Synthetic Chemistry
[b]E317 Institute of Lightweight Design and Structural
[c]Austrian Cluster for Tissue Regeneration

INTRODUCTION

The number of patients with minor and major accidents coming into accident and emergency care is steadily rising due to increasing life spans and the aging of our society. In order to ensure efficient care and health of human beings, medical methods need to be optimized. The fixation and adhesion between tissues, implants or scaffolds have to be refined, but the number and versatility of biomimetic and biocompatible adhesives that can be used for such purpose is limited. Up to now, adhesives based on cyanoacrylates, polyurethanes, epoxy resins or poly(methyl methacrylates) still bear several drawbacks ranging from possible allergic response, lack of mechanical strength to toxic side products. Therefore, new biomimetic glues for bonding of tissue-tissue and tissue-implant interfaces are urgently needed.

MATERIALS AND METHODS

A major challenge in determining the adhesion properties of a putative bone glue in a macroscopic setting is to distinguish between cohesive and adhesive forces. One approach circumventing this problem is to measure the adhesion via single molecule force spectroscopy (SMFS) referring to Lee *et al*.[1] This approach allows further to directly investigate adhesion processes at molecular level.

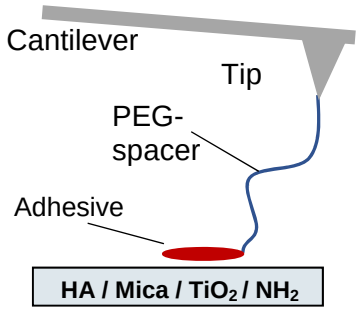

Figure 1: Adhesion measurement

Witin this study this approach was followed and a procedure was established to graft an adhesion motif onto the tip of an AFM cantilever via a linker system. In SMFS experiments the AFM tip works as a force sensor with pN resolution (see Figure 1).

In order to validate our approach, we decided to compare adhesion values with literature.[1] Therefore, the same dopamine-thiol compound as used by Lee *et al*. was attached to the AFM tip, adhesion force was measured and compared to published values. The hypothesis of this study was that the amino acid sequence D(pS)(pS)EEKC provides a strong and specific adhesion motif for hydroxyapatite, which is the organic component of bone. The specific amino acid sequence was chosen based on its suggested binding properties to hydroxyapatite in statherin[2] and due to similar sequences existing in non-collagenous proteins in bone, which are thought to be adhering to hydroxyapatite. We further hypothesised that the phosphorylated serines (pS) were largely responsible for the high interaction to Ca^{2+} in hydroxyapatite. In order to investigate this, an additional amino acid sequence with serines (not being phosphorylated) DSSEEKC was selected and tethered to an AFM tip. This allowed comparison of adhesion of the amino acid sequence with and without phosphorylation.

In the last few years studies of phosphorus-based polymers have attracted attention because of their large variety of applications.[3] In this study, we additionally investigated such polymers with regards to their adhesion properties. Therefore, we synthesized phosphor-containing methacrylates.[3] These monomers can bind to hydroxyl, carboxylic or amino groups of the organic collagen of a bone and form complexes with Ca^{2+} ions in the inorganic components. The synthesized phosphor-containing methacrylates were polymerized via reversible addition-fragmentation chain transfer (RAFT) polymerization. In this study, we prepared block copolymers with different molecular weight of dimethyl(methacryloyloxyethylmethacrylate) (DMMEP) and hydroxyethyl methacrylate (HEMA). These block-copolymers were tethered to the AFM tip and the adhesion properties were studied via single molecule force spectroscopy. The higher the amount of DMMEP (more ester groups), the higher should be the interaction between polymer and surface.

RESULTS AND DISCUSSION

In order to validate tip functionalization and experimental SFMS procedures the adhesion measurement with the linker system, the mean adhesion values of dopamine-thiol on TiO_2 were compared with literature. The measured adhesion values were in the same range as reported by Lee *et al*.[1] The pull-off forces of the naked tip were significantly lower than of the linker functionalized tip, which leads to the conclusion, that the attachment of the linker system was successful. Furthermore, the mean adhesion values of D(pS)(pS)EEKC on hydroxyapatite and TiO_2 were significantly higher compared to the values of DSSEEKC. As a result it was proved that, the phosphorylated serines were indeed responsible for the higher adhesion force on hydroxyapatite and on TiO_2 as it was suggested in literature.[2]

The synthesized block copolymers had significantly higher pull-off forces than dopamine (reference) and the amino acid sequences. The block copolymers with more polyDMMEP showed higher adhesion forces than the ones with less polyDMMEP. Thereby the phosphorus ester plays an important role to adhere on these substrates.

CONCLUSION

By the establishment of a procedure to graft a linker system with dopamine-thiol on the tip of an AFM cantilever, it was possible to measure the same pull-off forces on several substrates similar to Lee *et al*.[1] Moreover, it was proven that phosphorylated serines play a decisive role in adhering on hydroxyapatite. This means that the D(pS)(pS)EEKC motif can indeed be considered as a fully biocompatible adhesion motif for a bone glue. In addition to that, the block-copolymers with a longer block-chain of polyDMMEP showed very high adhesion especially on hydroxyapatite and TiO_2. Further research is now required at larger length scales to elucidate the feasibility of adhesives based on these motifs.

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