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Osteogenic potential of a biosilica-coated P(UDMA-*co*-MPS) copolymer

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Copolymer carrier preparation and nanoindentation parameters

The P(UDMA-*co*-MPS) copolymer carriers were obtained by mixing equal amounts of urethane dimethacrylate (UDMA) and methacryloxypropyltrimethoxysilane (MPS) with a combination of benzoyl peroxide and photoinitiator. The mixture was cast to shape circular specimens (ca. 200 µm thickness), light cured (400-500 nm, 5 min), and heated (100 °C, 30 min).

To determine elastic modulus and Martens hardness, 20 indents per sample were taken with a Berkovich tip, with a minimum spacing of 50 µm, using a NanoTest Vantage platform (Micro Materials, Wrexham, UK). For the first contact, the indenter velocity was set to 0.2 μms^{-1} and the initial contact force was kept constant to 0.05 mN. A maximum load of 10 mN was applied, with a 0.5 mNs^{-1} loading/unloading rate. To minimize the influence of viscoelastic deformation, the loading history comprised a hold for 120 s at constant maximum load before unloading. During this hold, displacement data were collected to determine the creep response. In the unloading history a second dwell period (300 s) was used at 10 % of the maximum load to

assess the thermal drift of the system. The elastic modulus and the hardness were extracted from the unloading data.[S1]

Surface-functionalization of P(MMA-*co*-MPS) carriers

To generate a biosilica-coating, the P(MMA-*co*-MPS) carriers (and the PMMA control) were successively dipped into a solution of 120 µg/ml recombinant mature silicatein-α ($aa_{115–330}$; in phosphate-buffered saline [PBS]) and a solution of 1.0 mM orthosilicate (in 50 mM Tris-HCl, pH 7.4) for 12 and 2 h respectively. Orthosilicate was prepared from prehydrolyzed tetraethyl orthosilicate (TEOS) as described.[S2] In short, a stock solution of 5 mM TEOS was mixed in a 1:3 M ratio with 1 mM HCl for 30 min and subsequently neutralized.

For immunodetection of surface-immobilized silicatein, the PMMA and P(MMA-*co*-MPS) carriers were incubated for 90 min (RT) with silicatein specific primary antibodies (diluted 1:1,000 in 15% blocking solution [Roche Applied Science, Mannheim, Germany]), followed by an incubation with Cy3-labeled species-specific secondary antibodies (diluted 1:8,000; 90 min, RT) (Dianova, Hamburg, Germany).[S3] Then, the samples were inspected by a Zeiss 710 cLSM (Carl Zeiss GmbH, Göttingen, Germany), using the 543 nm line of the helium/neon laser.

Concurrently, the samples were analyzed by Fourier transform infrared spectroscopy with attenuated total reflection (FTIR-ATR; Thermo Nicolet Nexus spectrometer with Golden Gate ATR accessory; Thermo Fisher Scientific, Dreieich, Germany), and scanning electron microscopy (SEM; LEO Gemini 1530; Zeiss, Oberkochen, Germany).

Mineralization of osteoblastic SaOS-2 cells on P(MMA-*co*-MPS) carriers

To investigate the effect of the biosilica-coated P(MMA-*co*-MPS) carriers on mineralization of osteoblastic cells, HA formation of SaOs-2 cells was analyzed. For this purpose, SaOS-2 cells (ATCC HTB-85) were cultured in McCoy's Medium Modified, supplemented with 15 % fetal

calf serum in a humidified incubator at 37 °C and 5 % CO_2. For the experiments, cells were seeded in multiwell plates onto P(MMA-*co*-MPS) discs (or PMMA as control) with a thickness of 200 µm. Where indicated, the discs had been coated with biosilica via immobilized silicatein. Furthermore, where indicated, the medium was supplemented after 3 d with DAAG cocktail (10 nM dexamethasone, 50 µM ascorbic acid, and 5 mM β-glycerophosphate) to stimulate mineralization for 7 d as previously described.[S4] To study mineralization by cLSM, cells on discs were washed several times with PBS, fixed in 4% [w/v] paraformaldehyde (5 min, RT), and permeabilized with 0.2 % [v/v] Triton X-100 (5 min, RT). Then, cells were blocked with 1 % [v/v] bovine serum albumin (in PBS; 30 min) and stained with OsteoImage (HA stain; Lonza, Cologne, Germany). Finally, the cells were counterstained with rhodamine phalloidin (actin cytoskeleton stain; Life Technologies, Darmstadt, Germany) and DRAQ5 (nuclear stain; Biostatus Ltd., Shepshed, UK), according to the manufacturers' instructions. Micrographs were acquired on a Zeiss 710 cLSM. The argon laser line of 488 nm was used to excite OsteoImage, whereas the 543 nm and the 633 nm line of a helium/neon laser were used to excite rhodamine phalloidin and DRAQ5 respectively. Concurrently, to quantitatively assess mineralization by fluoroscopy, cells were washed several times with PBS and fixed in ethanol for 20 min. Following incubation with OsteoImage and further washing, fluorescence was quantified using a Varioskan plate reader (Thermo Fisher) at 492/520 nm (excitation/emission wavelengths).

Alkaline phosphatase activity of osteoblastic SaOS-2 cells on P(MMA-*co*-MPS) carriers

To investigate the alkaline phosphatase (ALP) activity, SaOS-2 cells were cultivated as described above on P(UDMA) or silanol-functionalized P(UDMA-co-MPS) carriers in medium supplemented with DAAG for 7 days. The carriers had been treated with silicatein/orthosilicate or remained untreated. For semi-quantitative analyses, an ALP kit (Sigma-Aldrich, Taufkirchen,

Germany) was used. Hence, the cells were fixed, incubated in the ALP staining solution (containing naphthol AS-BI phosphate and fast red violet LB salt) for 15 min (RT), and counterstained with hematoxylin for 2 min, according to the manufacturer's protocol. Subsequently, the stained cells were inspected by phase contrast microscopy (EVOS XL, Life Technologies). Concurrently, ALP activity was determined spectrophotometrically for quantitative analyses. Thus, the cells were lysed in 10 mM Tris, 1 mM $MgCl_2$, and 0.1 % (v/v) Triton X-100, followed by three freeze-thawing cycles (at -80 °C and 37 °C). After centrifugation (15,000 g, 15 min, 4 °C), the supernatants were collected and used to determine both protein concentrations (QuantiPro BCA Protein Assay Kit, Sigma-Aldrich) and ALP activity. The latter one was calculated as the rate of p-nitrophenol production from p-nitrophenyl phosphate substrate (pNPP; SIGMAFAST p-nitrophenyl phosphate tablets, Sigma-Aldrich). For this purpose, 200 µl of a pNPP solution (1.0 mg/ml pNPP, in 0.2 M Tris and 5 mM $MgCl_2$) were added to 5 µl of cell lysate. After incubation at 37 °C for 10 min, the reaction was stopped by addition of 50 µl of 3 M NaOH. Then, the amount of p-nitrophenol produced was determined spectrophotometrically (405 nm) based on a corresponding standard curve. To calculate the ALP activity, the amount of p-nitrophenol was normalized by the total protein content. Data are expressed as means ± SD of five replicates. Statistical differences between the samples were assessed by Student's t-test.

References

S1 (*a*) W. C. Oliver and G. M. Pharr, *J. Mater. Res*., 1992, **7**, 1564; (*b*) S. H. Lee, Y. Teramoto, S. Wang, G. M. Pharr and T. G. Rials, *J. Polym. Sci. B Polym*. *Phys*., 2007, **45**, 1114.

S2 (*a*) M. Wiens, X. Wang, H. C. Schröder, U. Kolb, U. Schloßmacher, H. Ushijima and W. E. G. Müller, *Biomaterials*, 2010, **31**, 7716; (*b*) F. Natalio, T. Link, W. E. G. Müller, H. C. Schröder, F. Z. Cui, X. Wang and M. Wiens, *Acta Biomater*., 2010, **6**, 3720.

S3 M. Wiens, M. Bausen, F. Natalio, T. Link, U. Schloßmacher and W. E. G. Müller, *Biomaterials*, 2009, **30**, 1648.

S4 M. Wiens, X. Wang, U. Schloßmacher, I. Lieberwirth, G. Glasser, H. Ushijima, H. C. Schröder and W. E. G. Müller, *Calcif. Tissue Int*., 2010, **87**, 513.