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Morphology of Osteoblasts Grown on Doped Diamond-Like Carbon Coatings- An Electron Microscopy Study

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INTRODUCTION: Diamond-like carbon (DLC) coatings can be produced by several methods, including plasma immersion ion implantation and deposition (PIII&D). PIII&D is a method that allows the uniform coating of objects with complex geometries and the production of surface gradients that are especially important for the adhesion of DLC on metallic substrates. Further, with PIII&D various dopants can be incorporated into the DLC layer to produce variations in the surface physicochemistry and hence to alter the biological response. We studied the effect of Si-, H-, N-, and TiO-doping of DLC on human osteoblast adhesion and morphology.

METHODS: DLC-coatings were deposited on TiAl6V4 substrates with turned surface finish ($R_a = 520$ nm) by an ECR-based PIII&D process [1]. Five DLC variants were produced: non-doped ("0"), silicon-doped ("Si"), hydrogen-doped ("H"), nitrogen-doped ("N"), and titanium oxide-doped ("TiO") DLC by adding corresponding dopant precursors. They were characterized by elastic recoil detection analysis (ERDA) and Rutherford backscattering (RBS), see Tab. 1. Human osteoblasts were isolated, seeded on the DLC-coated and uncoated TiAl6V4 substrates, and incubated as previously described [2]. After 4 h and 24 h the cultures were washed, fixed in 2% glutaraldehyde, postfixed in 1% OsO_4 and dehydrated in graded series of alcohol and HMDS. The cell morphology was examined with an environmental SEM equipped with a BSE detector for imaging of elemental contrasts.

Table 1. PIII&D precursor gases and resulting composition of the DLC coatings.

DLC type	Precursor gases	Composition (at.%) (ERDA/RBS)
0	Hydrocarbon, Ar	C: 69, H: 30
Si	Hydrocarbon, Ar, HMDS	C: 57, H: 30, Si: 10, N: 3
H	Hydrocarbon, Ar, H_2	C: 60, H: 40
N	Hydrocarbon, Ar, N_2	C: 75, H: 22, N: 3
TiO	Hydrocarbon, Ar, $TiCl_4$, CO_2	C: 40, H: 18, Ti: 19, O: 23

RESULTS: The SEM images revealed similar osteoblast morphology on the DLCs and the TiAl6V4 surfaces for both culture periods. The cells adhered readily on all surfaces after 4 h and exhibited a round shape with discrete cytoplasmic extensions at periphery. After 24 h the osteoblasts switched to the polygonal morphology with prominent cytoplasmic extensions (Fig. 1).

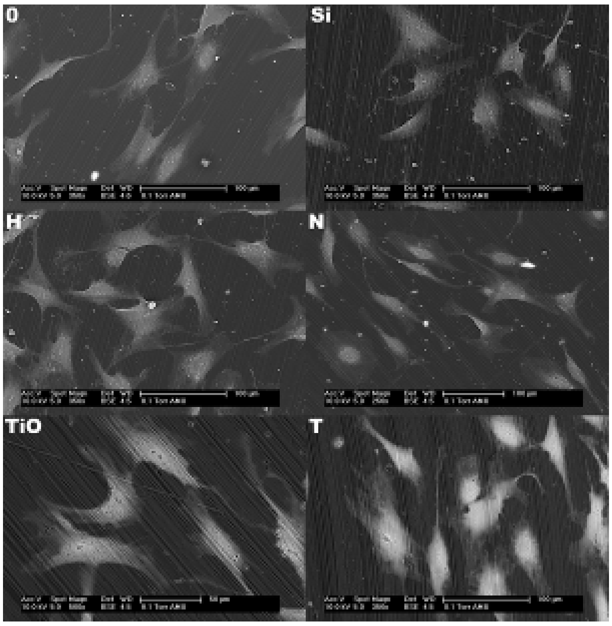

Fig. 1: Osteoblast morphology on the different DLC surfaces: non-doped (0), Si-doped (Si), H-doped (H), N-doped (N), TiO-doped (TiO), in comparison to TiAl6V4 (T) after 24 h in culture.

DISCUSSION & CONCLUSIONS: The successful osteointegration of an implant relies on its ability to support the building of bony tissue on its surface. In order to proliferate and subsequently produce ECM components, osteoblasts need to adhere and spread on the implant surface. Our results show that the selected DLC coatings are favorable for osteoblast adhesion and spreading similar to TiAl6V4.

REFERENCES: [1] G. Thorwarth, C. Hammerl, M. Kuhn, et al (2005) *Surf Coat Technol* **193**:206-212. [2] G. Thorwarth, B. Saldamli, F. Schwarz, et. al (in press) *Plasma Process Polym* DOI: PPAP 200731001 I.R.

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