Neutral atom beam technique enhances bioactivity of PEEK

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Abstract

Polyetheretherketone (PEEK) is currently gaining popularity in orthopedic and spinal applications but has potential drawbacks in use. PEEK is biocompatible, similar in elasticity to bone, and radiolucent; however, it has been shown to be inert and does not integrate well with bone. Recent efforts have focused on increasing the bioactivity of PEEK by modifying the surface to improve the bone-implant interface. We have employed a novel Accelerated Neutral Atom Beam technique (ANAB) to enhance the bioactivity of PEEK. ANAB employs an intense beam of cluster-like packets of accelerated unbonded neutral argon (Ar) gas atoms. These beams are created by first producing a highly energetic Gas Cluster Ion Beam (GCIB) comprised of van der Waals bonded Ar atoms, then transferring energy to the clusters so as to cause release of most of the interatomic bonds, and finally deflecting away the remaining electrically charged cluster cores of still bonded atoms. We identified that ANAB treatment of PEEK results in nanometer scale surface modifications as well as increased surface hydrophilicity. Human osteoblasts seeded onto the surface of ANAB-treated PEEK exhibited enhanced growth as compared to control PEEK as evidenced by cell proliferation assays and microscopy. This increase in bioactivity resulted in cell proliferation levels comparable to native titanium. An in vivo study using a rat calvarial critical size defect model revealed enhanced osseointegration where bone tissue formation was evident only on the ANAB treated PEEK. Taken together, these data suggest that ANAB treatment of PEEK has the potential to enhance its bioactivity, resulting in bone formation and significantly decreasing osseointegration time of orthopedic and spinal implants.

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1. Introduction

Polyetheretherketone (PEEK), a dominant member of the Polyyetheretherketone (PAEK) family, has been successfully used in the manufacturing and use of spinal, orthopedic, and trauma implants for over 25 years [1–4]. A key benefit for the use of PEEK in medical devices has been its radiolucency; its ability to be imaged by X-ray, CT scan, or MRI without distorting the visualization of the desired fusion as compared to the traditional titanium (Ti) and stainless steel materials used in these applications [5,6]. PEEK’s chemical resistance and stability also make it a material of choice for long term use in the body without any breakdown products [5,6]. Another attractive property of PEEK is the ability to manipulate its elastic modulus to more closely match that of other materials [7,8]. The addition of carbon or glass can increase the modulus from 3–4 GPa to 18 GPa to mimic bone, or 150 GPa to mimic Ti [4,9]. A drawback of PEEK, on the other hand, is that due to its inert properties, PEEK fails to integrate well with bone [1,10–12]. Initially, this has been overcome with the addition of growth factors and proteins such as BMP-2 [13]; however as complications and issues are starting to arise surrounding the use of BMPs [14], there is an unmet need to create a more bioactive PEEK. Many groups are currently attempting to increase PEEK’s bioactivity by adding bioactive compounds such as hydroxyapatite [15–19], calcium phosphate (CaP) [20], Ti [16,21], and others [11,22,23]. Various groups have attempted to use oxygen plasma to increase surface energy [12,24], and others are working on making porous PEEK to allow cellular ingrowth [25]. All these methods have had limited in vitro success.

We have developed a novel accelerated neutral atom beam technology (ANAB) that can modify the surface of implantable medical devices to a shallow depth of no more than 5 nm. The ANAB surface modification technique, which is described in detail elsewhere [26], employs intense directed beams of neutral gas atoms having average energies which can be controlled over a range from a few eV per atom to beyond 100 eV per atom. These neutral atom beams are created by dissociating energetic gas cluster ions produced by the Gas Cluster Ion Beam (GCIB) technique [27]. A beam of energetic gas cluster ions is created by expanding a gas through a small nozzle into vacuum to form a stream of weakly-bonded gas clusters, then ionizing the clusters by electron impact and accelerating them...
through a high potential. Energy is then transferred to the clusters by means of gas atom collisions caused to take place immediately after acceleration, making the clusters unstable and forcing them to release large numbers of their constituent neutral atoms. Released neutral atoms then continue to travel collectively with the same velocities they had prior to being released. An electrostatic deflector is used to eliminate all residual charged species from the beam. Monitor assemblies to measure atom flux and beam power are used for characterization and control.

The goal of this study was to determine if ANAB surface processing could enhance the bioactivity of PEEK without modification of surface chemistry and without the addition of bioactive substances. Surface modification by ANAB was studied using both atomic force microscopy and by assessing contact angle. Osteoblast adhesion and proliferation were investigated in vitro on control and ANAB treated PEEK and Ti. In addition to the proliferation studies, in vivo osseointegration of ANAB treated PEEK was studied in a rat calvarial defect model.

2. Materials and methods

2.1. Treatment of surfaces by neutral atom beam technique (ANAB) and analytical measurements

PEEK film 0.2 mm thick (McMaster Carr) and titanium (Ti) foil 0.1 mm thick (Ti, CP1, Alfa Aesar) were cut to 1 cm diameter; PEEK rods 3 mm diameter were cut to 1 mm thick disks. Materials were cleaned in 70% isopropanol for 30 min followed by 3 × 5 min washes in H2O. All materials were prepared as controls or treated by ANAB using Argon (Ar) gas on an accelerated particle beam system (nAccel 100, Exogenesis Corp) with a deflector to remove charged clusters. Accelerated neutral atoms were produced from GCIB cluster ions accelerated through 30 kV. The measured average energy of the accelerated neutral atoms was approximately 40 eV/atom. The distribution of individual atom energies could not be measured directly, but was believed to range from a minimum of a few eV/atom to a maximum of 80 eV/atom. The effective dose

Fig. 1. ANAB results in nano-scale texturing of the surface of PEEK. AFM measurements revealed the generally smooth surface of PEEK (a) results in nano-texturing following ANAB treatment (b). ANAB also results in increased PEEK surface hydrophilicity as measured by water contact angle. Control PEEK surface (c) has a contact angle of θ = 76.4° while ANAB-treated PEEK has a contact angle of θ = 36.1°.
of the Ar ANAB was $2.5 \times 10^{17}$ atoms per cm$^2$. AFM measurements were taken using a Park Systems XE-70 instrument in non-contact mode. Silicon tips with a resonant frequency of $\approx 330$ kHz and a force constant of 42 N/m were used (PointProbe$^\text{®}$ Plus, Nanosensors). 1 µm regions of the PEEK were imaged and the arithmetical mean roughness ($R_a$) and ten-point mean roughness ($R_z$) was measured across this region. Contact angle was measured using the sessile drop method on a manual simplified device as described by Lamour et.al. (2010) [28] and droplet angles were measured by ImageJ software (NIH) with the contact angle plugin.

2.2. Cell attachment and proliferation

Human fetal osteoblast cells (hFOB 1.19, ATCC) were maintained in Dulbecco’s modified Eagle Minimum Essential Medium (DMEM/F12, Life Technologies) containing 10% fetal bovine serum (FBS, Life Technologies) in a humidified incubator at 37 °C, 5% CO2, 95% air. PEEK film ($n = 3$ per condition and time) were placed in 24 well dishes and 2000 hFOB were seeded on the surface in 1 ml medium and allowed to attach and proliferate for up to 14 days. In a parallel study, ANAB-treated and control PEEK were compared to control Ti over a 10 day study. Cell counts were performed using the MTS assay (Promega) or crystal violet assays (Sigma) and measured against a standard curve on a plate reader.

2.3. Rat calvarial defect study (in vivo)

All animal work was approved and performed at CBSET (Lexington, MA) following a local ethical review. CBSET is registered with the United States Department of Agriculture (USDA) to conduct research in laboratory animals. All procedures and conditions of testing complied with the Animal Welfare Act and its amendments. Male Sprague–Dawley rats, $\approx 250$ grams ($n = 6$ per condition) were anesthetized with isoflurane gas and the surgical site was shaved. A small incision was made in the top of the skull, and the skin and underlying tissue were incised and reflected to expose the cranium. A drill with a 3 mm burr was used to create the defect site, using care to avoid damaging the dura mater. One implant (either a test or control device) was placed in the defect site, and the soft tissue and skin was closed appropriately in layers. Moribundity/mortality checks were performed twice daily and all animals in the study performed well. Four weeks after implantation, animals were sacrificed and the cranium was removed in toto and placed in 10% buffered formalin. Undecalcified, intact calvaria with implant sites were resin embedded and micro-ground and histomorphologic evaluation was used to qualitatively assess and characterize the extent of new bone deposition and relative to the surface of the implant.

3. Results and discussion

3.1. ANAB treatment results in surface modifications of PEEK

AFM measurements revealed a nanometer (nano) scale texturing of the surface on ANAB-treated PEEK film as compared to that of native Ti. hFOB cells growing on surfaces of ANAB-treated PEEK display rapid proliferation as compared to control surfaces. ANAB results in increasing the bioactivity of PEEK, comparable cell attachment and proliferation is seen on bioactive Ti. There were no significant differences in cell proliferation between the Ti and ANAB-treated PEEK groups at any time point.
controls (Fig. 1a and b). The level of texturing has been determined to be in the range of 10–50 nm. Several groups have indicated the importance of surface nano-texturing on cell attachment and proliferation. Although various methods currently exist for nano-scale texturing on metals, alloys, and certain polymers including sintering [29,30], chemical vapor deposition [31,32], acid etching [33], and lithography [34], none of these methods would work effectively on chemically resistant PEEK [4]. Bombardment of the surface with Ar atoms results in a controllable and reproducible texturing in line with other methods. ANAB-treatment of the surface resulted in decreased Ra from 12.5 nm on control to 10.9 nm on treated PEEK. Rz measurements also decreased from 93.1 nm on control to 83.8 nm on ANAB-treated PEEK. These changes are consistent with a nano-scale modification of the material surface. In comparison of the surfaces, we identified that hydrophilicity has increased on ANAB-treated PEEK as seen by decreased water contact angle from $\theta = 76.4^\circ$ on control to $\theta = 36.1^\circ$ on ANAB-treated PEEK (Fig. 1c and d; $p < 0.05$). Surface hydrophilicity is known to be important for initial cell attachment [35–37]. By increasing the hydrophilicity on PEEK, more cells may attach at an earlier period on ANAB-treated cells as compared to untreated controls.

### 3.2. ANAB treatment results in enhanced bioactivity of PEEK

In order to identify if the surface modifications have changed the ability of PEEK to enhance cell attachment, proliferation, and overall bioactivity, we have designed cell based studies that compare ANAB-treated PEEK to controls. In the first set of cell culture studies, we assayed whether osteoblast cells could be made to attach and proliferate on PEEK. Due to its inert properties, it has been well documented that PEEK is not a suitable material for osteoblast proliferation. PEEK film that was treated by ANAB processing was compared to controls. hFOB cell proliferation on these surfaces was observed at 1, 3, 7, and 14 days post seeding using crystal violet dye for visual inspection by microscope and for measurement of dye elution as compared to a standard curve. Although very few cells appeared to attach on control PEEK films at day 1 (data not shown) the cells either delaminated or failed to proliferate by day 14 (Fig. 2a and c). In comparison, hFOB cells seeded onto ANAB-treated PEEK resulted in very good cell attachment and proliferation over the 14 day period (Fig. 2b and d). Seeing that osteoblasts were able to attach and proliferate on the ANAB-treated PEEK, we next attempted to find if this treatment increases PEEK’s bioactivity in comparison to native Ti, which is considered to be bioactive. Osteoblasts growing on ANAB-treated PEEK resulted in similar cell attachment and proliferation as compared to native Ti over the 10 day period. Osteoblasts growing on ANAB treated PEEK significantly increased as compared to untreated PEEK ($p < 0.02$ at day 7 and 10). Cell proliferation on ANAB-treated PEEK, which were initially seeded at 2000 cells, proliferated to 7830 ± 700 at day 10 while cells growing on control PEEK maintained cell viability without any appreciable proliferation reaching 2675 ± 278 cells ($p < 0.003$, Fig. 3). Osteoblasts growing on native Ti foil reached 6,822 ± 422 cells at day 10 which represents no significant difference from ANAB-treated PEEK ($p = 0.2$).

While Ti had been the favored material in spinal fusions due to its bioactivity and its ability to promote fusions, its inability to allow the fusion to be clearly imaged by X-ray or other means eventually caused it to fall out of favor in comparison to PEEK. Another reason to switch to PEEK is that the elastic modulus of Ti is much greater than cortical bone ~150 GPa versus ~18 GPa respectively [4,9]; this difference of stiffness could result in bone resorption around the implant. The downside to PEEK, however, is its inert characteristic results in poor to no osseointegration. A way to improve its ability to integrate has been the use of bone morphogenetic protein [13], which in recent times has resulted in poten-

![Fig. 4. ANAB enhances bioactivity of PEEK and results in bone integration in vivo. Rat calvarial defect study reveals control PEEK has very little ability to integrate with surrounding bone. Only a fibrous layer is seen by histopathological methods on control PEEK disks, further bone resorption is evident on the edges where bone makes initial contact with PEEK (a). ANAB-treated PEEK disks (b) results in very good purchase on the contact site between bone and PEEK as well as a cortical bone ledge that appears to cover nearly 50% of the surface by week 4 (indicated by arrows).](image-url)
tional complications associated with overuse and has grown out of favor with orthopedic surgeons [14]. Many methods to enhance the bioactivity of PEEK are currently being studied, including the addition of bioactive molecules such as calcium phosphate (CaP) [20], hydroxyapatite [15,18], and even Ti [16,21]. The addition of these molecules has resulted in limited success in vitro.

In order to determine if ANAB-treatment could enhance the ability of PEEK to integrate with bone, we have performed a rat calvarial critical size defect model comparing treated to untreated PEEK disks. Following 4 weeks after implantation into the calvarial defect, histology was performed to determine the amount of bone regrowth on the surface. We identified that ANAB-treated PEEK resulted in a bone ledge growing on the top of the disk covering approximately 50% of the surface whereas the control PEEK resulted in only fibrous tissue with no bone growth at all (Fig. 4). The controls displayed the start of bone resorption on the sides of the disks whereas good bone purchase is seen on the ANAB-treated disks. This in vivo model has proven that ANAB treatment of otherwise inert PEEK can increase its bioactivity and its ability to osseointegrate in the bone.

4. Conclusions

We have developed a novel accelerated neutral atom beam technique that could modify the surfaces of implantable medical devices to enhance surface bioactivity. ANAB which employs intense directed beams of neutral gas atoms having average energies which can be controlled over a range from a few eV per atom to beyond 100 eV per atom results in a controllable nanometer scale texturing of the surface to a depth of no more than 5 nm. The resultant texture which has peak diameters of 10–50 nm is favorable for cell attachment and proliferation. By using Ar gas in the process of ANAB treatment, we are not changing any of the surface chemistry; we have developed a novel accelerated neutral atom beam technique to enhance the surface characteristics of PEEK.

References


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