Morphological Characterization of Polyanhydride Biodegradable Implant Gliadel® During *in Vitro* and *in Vivo* Erosion Using Scanning Electron Microscopy

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Purpose. The objectives of the current study are to characterize the distribution of the chemotherapeutic agent carmustine (BCNU) in spray dried polyanhydride microspheres and to describe the morphological changes that occur during the *in vitro* and *in vivo* erosion of the polyanhydride implant—GLIADEL®, which consists of BCNU distributed in the copolymer matrix of poly(carboxyphenoxy propane:sebacic acid) in a 20:80 molar ratio (p(CPP:SA, 20:80)).

Methods. Scanning electron microscopy (SEM) was used to visualize the morphological changes of the polymer during the manufacturing process and *in vitro* and *in vivo* erosion.

Results. This study revealed that BCNU was homogeneously distributed within spray dried polyanhydride microspheres with no phase separation. The porosity of the wafer fabricated from spray dried polyanhydride microspheres gradually increased during erosion. During the initial period following wafer implantation in the brains of rats, erosion was mainly confined to the surface layer of the wafer with the majority of the wafer remaining intact. The eroding front gradually advanced from the surface to the interior of the wafer in a layerwise fashion, creating pores and connecting channel. Eventually both the interior and exterior of the wafers were eroded and the same porous structure was seen throughout the whole wafer.

Conclusions. This study provides the first visual observation of the morphological changes of the GLIADEL® wafer during erosion of the polyanhydride matrix and release of the drug substance BCNU. The observations in this study support the conclusion that BCNU release from a polyanhydride wafer is controlled both by diffusion of the drug and erosion of the polymer matrix.

KEY WORDS: controlled release; polyanhydride; biodegradable polymer; brain tumor; GLIADEL® implant; scanning electron microscopy.

INTRODUCTION

Traditional methods for delivering chemotherapeutic agents to the brain are severely limited because of the existence of the blood-brain barrier which prevents most drugs from entering the intracranial tissue via blood circulation. Direct implantation of drug-containing polymer pellets at the desired location in the brain bypasses the blood-brain barrier and offers several advantages over conventional treatment modalities, including higher bioavailability and reduced systemic toxicities. Among current synthetic biodegradable polymers designed for controlled release drug carriers, polyanhydrides are one of the few that are being tested as implants in humans (1–3). The GLIADEL® wafer is a biodegradable polyanhydride polymeric matrix impregnated with the active ingredient carmustine (BCNU) that can provide targeted and controlled release of BCNU after implantation in the tumor cavity. GLIADEL® wafer has shown to be a safe and effective treatment for malignant gliomas in randomized, double-blind, placebo-controlled, multi-center human Phase III clinical trials (2, 4). The formulation, production, sterilization, and stability of the wafer have been described by Chasin et al. (5). However, no thorough investigation has been undertaken to characterize the distribution of BCNU in the GLIADEL® wafer and the morphological changes of the wafer during *in vitro* and *in vivo* degradation.

The chief objective of the current study is to characterize, by scanning electron microscopy (SEM), the morphological changes of the p(CPP:SA, 20:80) polymer during the manufacturing process of GLIADEL® wafers and during *in vitro* and *in vivo* erosion of the polymer. SEM is a very powerful tool for analyzing the erosion of polymeric drug delivery systems (6) and the effects of microsphere fabrication methods on polymer erosion and drug release (7–9). In the current study, SEM was used to visualize the morphological changes of the polymer matrix during manufacturing, and *in vitro* and *in vivo* erosion processes. The results obtained provide a clear understanding of the mechanisms of polymer erosion and drug release from GLIADEL® wafers.

MATERIALS AND METHODS

Preparation of GLIADEL® Wafers

Sebacic acid, 4-hydroxybenzoic acid and acetic anhydride were purchased from Aldrich Chemicals (Milwaukee, WI). Carmustine (BCNU) was purchased from Aerojet (Sacramento, CA). Poly[bis(p-carboxyphenoxy)propane-*co*-sebacic acid] P(CPP:SA, 20:80) was synthesized by melt polycondensation (10). P(CPP:SA, 20:80) melts were precipitated in a 1:1 ether:petroleum ether mixture from a 20% methylene chloride solution and vacuum dried. The precipitated materials were further dissolved in methylene chloride (5%) and the solution was sprayed using a Büchi Model B-191 mini spray drier (Brinkmann, Westbury, NY) to evaporate the methylene chloride. To incorporate BCNU into the polymer matrix, an appropriate amount of BCNU was dissolved in the 5% polymer solution and spray dried. The spray dried microspheres were compressed into 200 mg wafers by using a Model C Carver Press (Carver Inc., Wabash, IN). The chemical structures of BCNU and p(CPP:SA, 20:80) are shown in Figure 1.

Studies of Wafer Erosion

Small wafers (~ 13–15 mg, 3 mm in diameter, 1 mm in thickness for *in vivo* study and ~ 30 mg, 5 mm in diameter, and 1 mm in thickness for *in vitro* study) were cut from the 200 mg wafer by using a cork borer, and individually weighed. *In vitro* erosion was studied by incubating the wafers in phosphate buffered saline (PBS, pH = 7.4) at 37°C under constant

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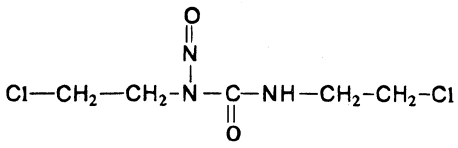
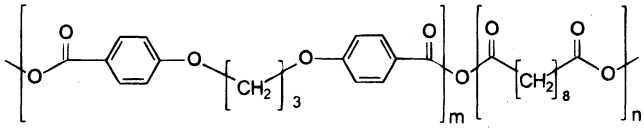


1,3-bis(2-chloroethyl)-nitrosourea (BCNU)



P(CPP:SA, 20:80); m=20 and n=80.

Fig. 1. The chemical structure of polyanhydride copolymer p(CPP:SA, 20:80) and drug substance carmustine (BCNU).

shaking at 110 rpm. At 2, 4, 8 hours and 1 day following the incubation, the wafers were retrieved from the solution and lyophilized on dry ice overnight. The lyophilized wafers were then characterized by SEM. For *in vivo* erosion studies, implantation of GLIADEL® wafers was accomplished by inserting the wafer into a cortical defect surgically created in the brain of a normal rat (Fisher 344) as described by Reinhard et. al. (11). All surgical procedures adhered by the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985). At 2 hours, 1 and 5 days following wafer implantation, implanted wafers were surgically retrieved from sacrificed rats. Wafers were immediately frozen on dry ice and then lyophilized overnight to constant weights. The morphology of the wafer was then characterized by SEM.

Determination of Polymer Molecular Weight

The molecular weight of the each sample was determined by gel permeation chromatography (GPC). Briefly, samples were dissolved in methylene chloride and 20 ml of each polymer solution were injected into two serial Polymer Labs PLgel Mixed C columns (300 × 7.5 mm, 5 μm particle size) in a Waters Model 510 chromatographic system. A Waters Model 410 Refractive Index detector was used for detection. The compounds were eluted using methylene chloride with a flow rate of 1.0 ml/minute (column temperature at 30°C and detector temperature at 35°C). A Waters 840 Data system was used for data acquisition and analysis. Monodispersed polystyrene standards were used to calibrate the GPC column.

Morphological Characterization of the Wafer

Morphology of the surface and cross sections of wafers was examined using a scanning electron microscope (Model JEOL 6400). Spray dried microspheres were similarly characterized. Wafer cross sections were obtained by flexure of the samples after immersion in liquid nitrogen. To obtain the crushed microspheres, spray dried microspheres were wrapped in aluminum foil and crushed in liquid nitrogen. All the samples were mounted on metal stubs and sputter-coated with a 200–300 Å layer of gold before examination.

RESULTS

Morphological Characterization of Spray Dried Microspheres and Wafers

Examination by SEM showed that polymer powders obtained by the spray drying process were a cluster of aggregated microspheres with a particle size range of approximately 1–20 μm (Figure 2A&B). Microspheres with no incorporated BCNU were spherical with a smooth external surface and some pores on the surface (Figure 2A). When BCNU was added to the polymer solution, the external surface of the spray dried drug-containing microspheres remained smooth with no visible pores on the external surface (Figure 2B). When crushed microspheres were examined under SEM, no precipitated or crystallized phase was observed within the microspheres. The interior and exterior of the BCNU-containing microsphere appeared to be a single smooth, homogeneous phase, suggesting that BCNU was uniformly distributed without disrupting the continuity of the polymer phase (Figure 2C). When the BCNU-containing microspheres were analyzed by differential scanning calorimeter (DSC), a single melting peak was observed with a maximum melting point of approximately 58°C, which is between those of pure BCNU (~32°C) and p(CPP:SA, 20:80) (~65°C) (unpublished data). As the BCNU content in polymer matrix increased, the melting point of wafer decreased (unpublished data). All these results further support the observations by SEM that BCNU was distributed in the polymer phase as a solid solution.

After the compression molding process, the resultant wafer surface appeared very uniform, with spray dried microspheres densely packed together (Figure 3A). When a cross section of the wafer was examined, individual microspheres were clearly identifiable along with very small gaps among them (Figure 3B). These gaps may create inter-connecting channels for water to penetrate from the exterior to the interior of the wafer during the erosion process.

Morphological Changes of the Wafer During *in Vitro* Erosion

Wafers with two different initial molecular weights (~72 kDa and ~48 kDa) were examined by SEM for *in vitro* erosion. In general, the surface porosity of a wafer increased as it degraded in PBS (Figure 4). Major changes in surface porosity occurred during the first day of incubation. For 72 kDa wafers, the wafer surface was not porous at the end of a 2 hour incubation period, and individual microspheres could be seen clearly under SEM (Figure 4A). After 4 hours of incubation, individual microspheres were not well defined but could still be identified (Figure 4B). After 1 day of incubation, only small polymer pieces were observed, with very porous surfaces (Figure 4C). For 48 kDa wafers, although the trend in surface morphology change was similar to that of 72 kDa wafers, the wafer surface became porous at an earlier time. For instance, after 2 hours of incubation, although individual microspheres were clearly visible, the surface of each microsphere became very porous, indicating the erosion of polymer had started (Figure 4D). After

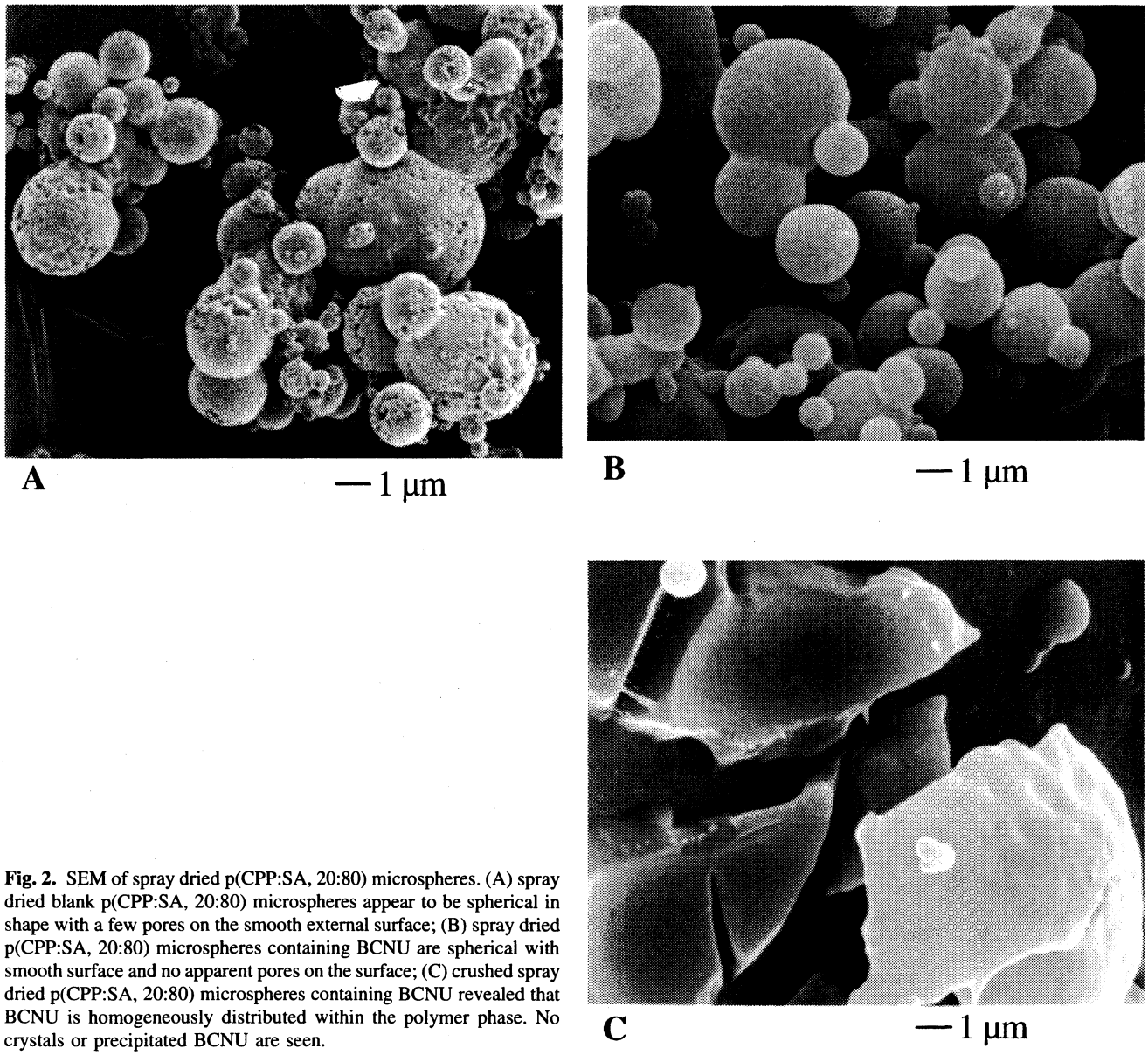


Fig. 2. SEM of spray dried p(CPP:SA, 20:80) microspheres. (A) spray dried blank p(CPP:SA, 20:80) microspheres appear to be spherical in shape with a few pores on the smooth external surface; (B) spray dried p(CPP:SA, 20:80) microspheres containing BCNU are spherical with smooth surface and no apparent pores on the surface; (C) crushed spray dried p(CPP:SA, 20:80) microspheres containing BCNU revealed that BCNU is homogeneously distributed within the polymer phase. No crystals or precipitated BCNU are seen.

8 hours of incubation, individual microspheres were very difficult to identify and the gaps among individual microspheres greatly diminished (Figure 4E). By the end of the first day of incubation, individual micropsheres could no longer be seen and the surface of the wafer appeared to be very porous, similar to that of a 72 kDa wafer at 1 day of incubation (Figure 4F).

Morphological Changes of the Wafer During *in Vivo* Erosion

Examination of the wafer by SEM before and after implantation in rat brains clearly demonstrated the morphological changes of the wafer during erosion and release of BCNU. Before implantation, the surface of a BCNU-loaded wafer appeared very uniform, with spray dried microspheres densely packed together on the outer surface (Figure 3A). Two hours after the implantation, the surface layer of the wafer was eroded with the next layer of compressed microspheres clearly identifiable (Figure 5A). One day following implantation, the wafer surface became very porous and no individual microspheres could be identified under SEM (Figure 5B).

When the cross sections of the degrading wafer were examined under SEM, the dynamic process of water penetration from the surface to the interior was apparent (Figure 6). Two hours after the wafer implantation, the porous structure extended approximately 20–30 μm from the surface into the interior of the wafer (Figure 6B). The majority of the BCNU-incorporated wafer still remained intact, with individual microspheres clearly visible under SEM (Figure 6C). One day following the implantation, however, the bulk of the wafer was eroded with the characteristic porous morphology extending approximately 100 μm into the interior from the surface. Nevertheless, closer to the

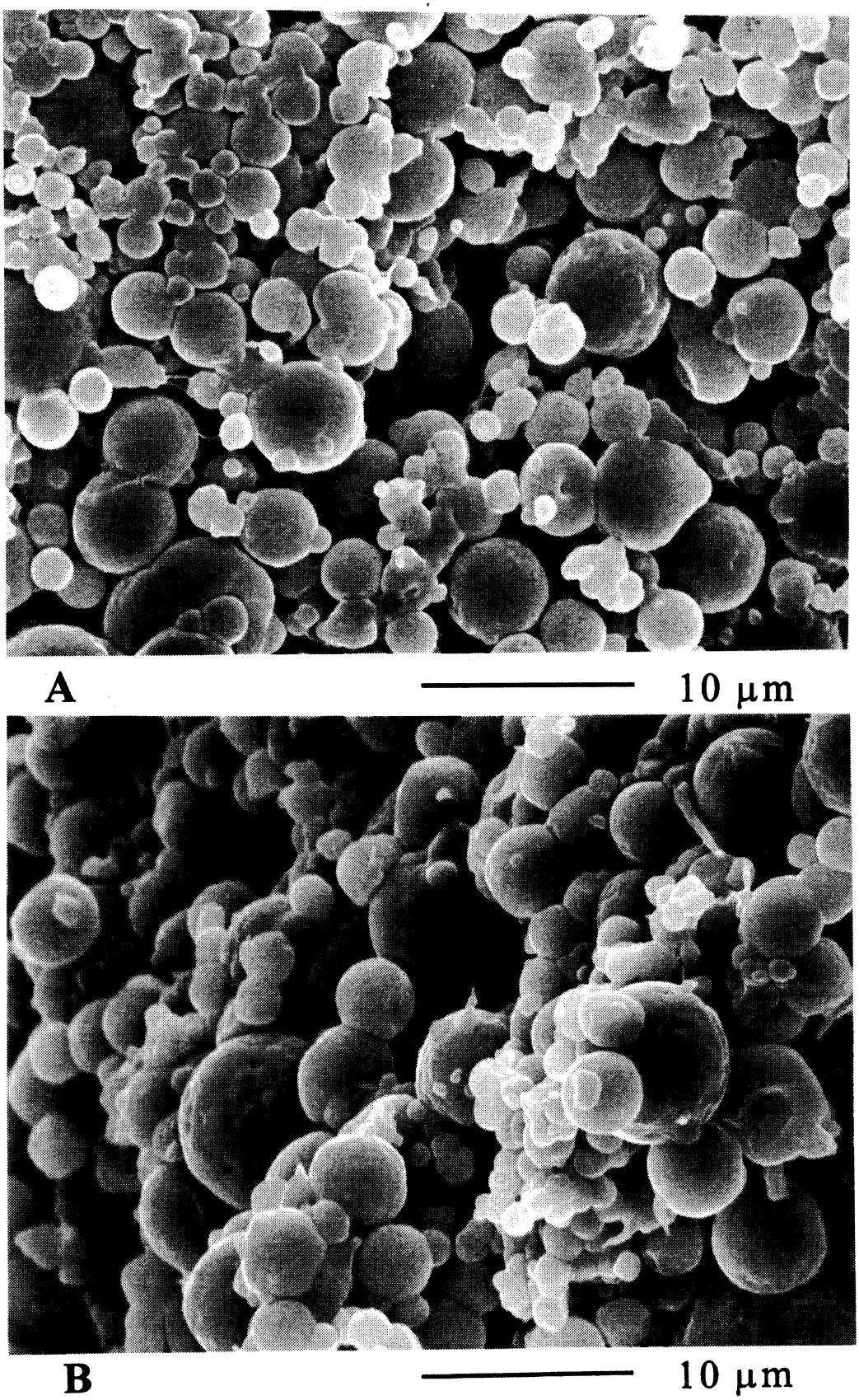


Fig. 3. SEM of GLIADEL® wafer. (A) The surface morphology of the wafer. Spray dried microspheres are densely packed together. (B) Cross section of the wafer. Individual microspheres are identifiable.

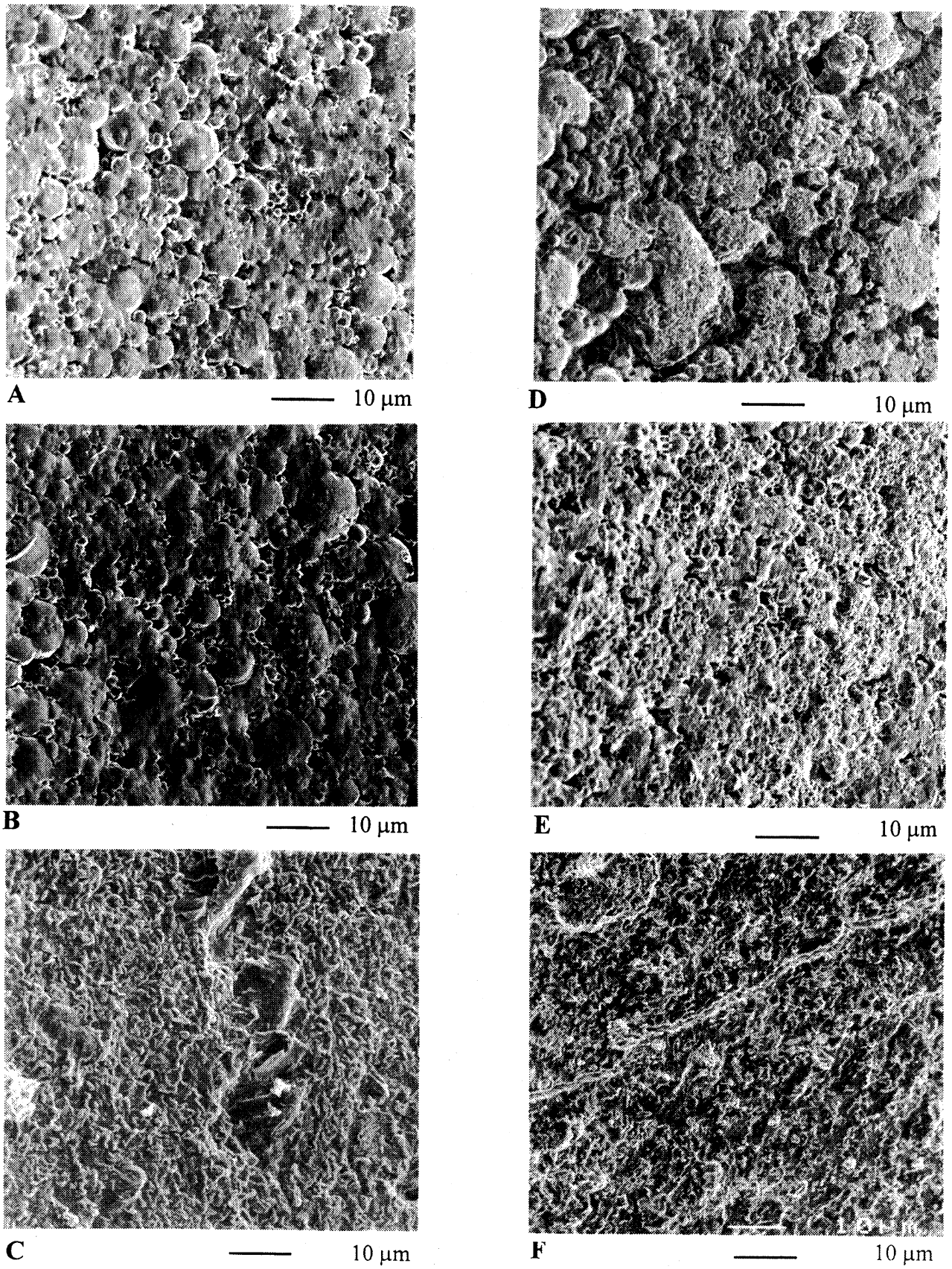
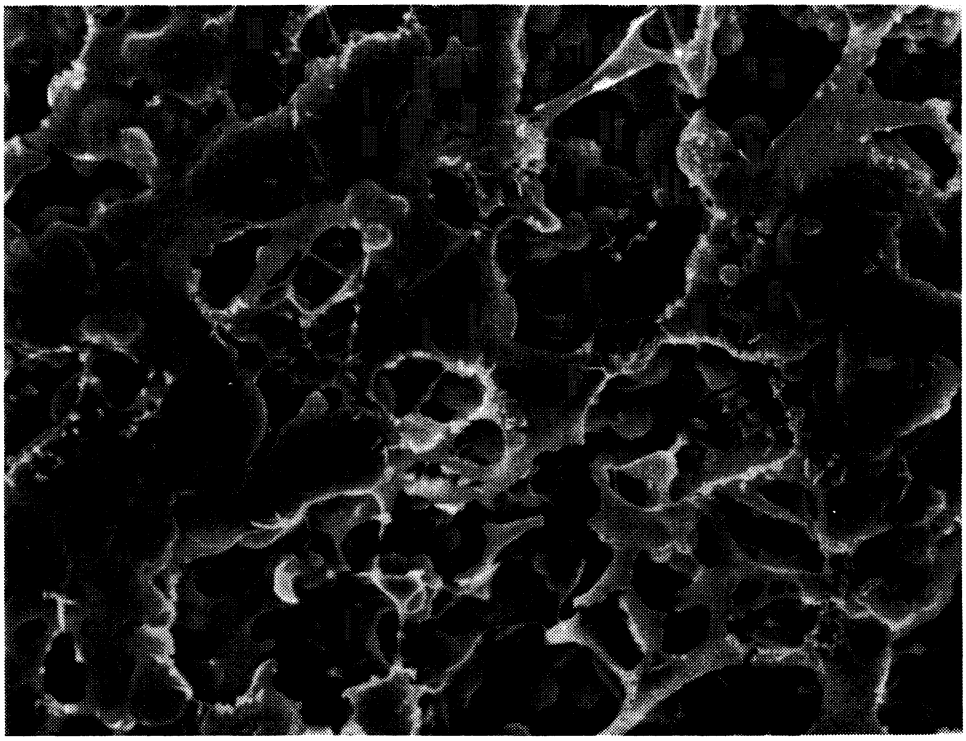


Fig. 4. SEM of blank p(CPP:SA, 20:80) wafer surface at various stages of incubation in PBS at 37°C. (A) to (C) are wafers with a initial molecular weight of 72 kDa; (D) to (F) are wafers with a initial molecular weight of 48 kDa. (A) Two hours; (B) Four hours; (C) One day; (D) Two hours; (E) Eight hours; (F) One day.



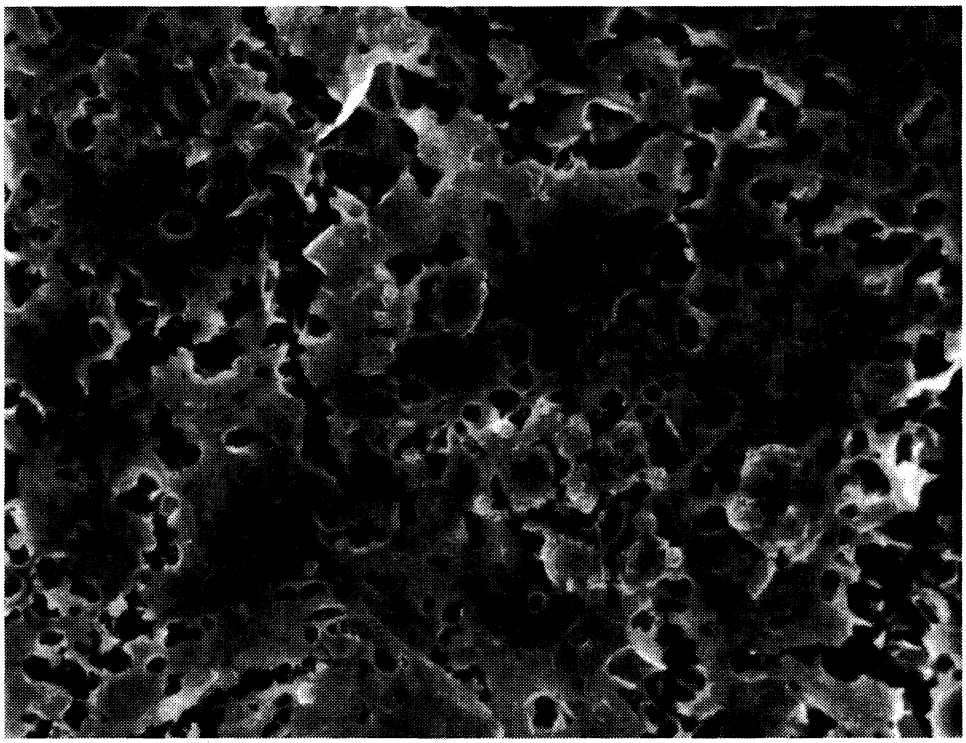
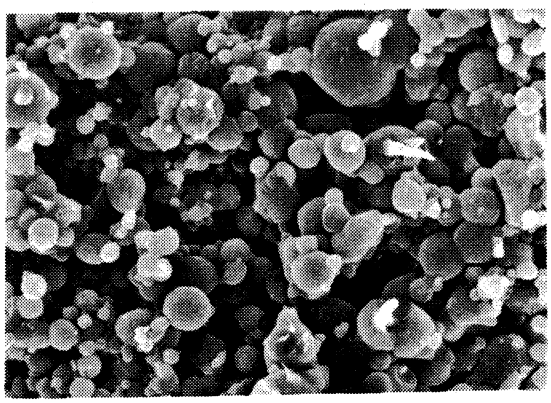
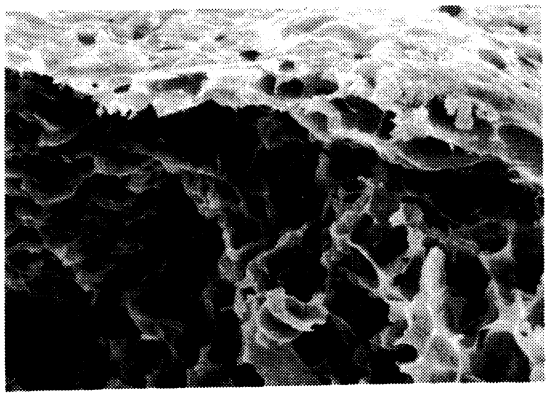


Fig. 5. SEM of GLIADEL® wafer surface after implantation in the brains of rats for various periods of time. (A) Two hours after the wafer implantation, the surface layer of the wafer was eroded with deeper layers of compressed microspheres clearly visible; (B) One day after the implantation, the wafer surface became very porous and no individual microsphere could be identified.

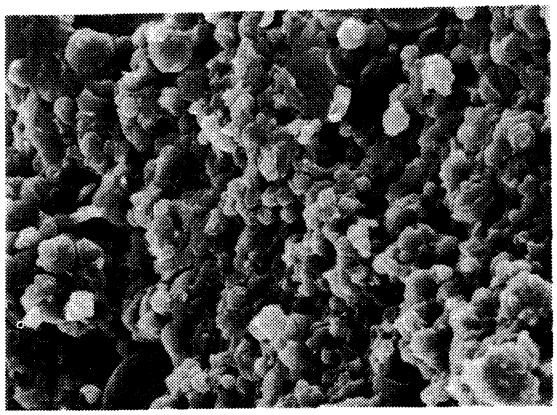
Fig. 6. SEM of GLIADEL® wafer cross-section before and after implantation in the brains of rats for various periods of time. (A) Cross-section of wafer before implantation; (B) Cross section immediately beneath wafer surface at two hours after wafer implantation. The structure was porous, similar to the surface; (C) Bulk portion of wafer cross section at two hours after wafer implantation. The microspheres remained intact, individual microspheres were clearly visible; (D) Cross section of wafer at one day following wafer implantation. Approximately 100 μm beneath wafer surface was porous (eroding zone) with the core of the wafer remaining intact; (E) Higher magnification of the eroding zone. Large portions of the wafers were occupied by pores and connecting channels with some intact microspheres; (F) Higher magnification of an eroded microspheres; (G) Five days following the implantation, the entire wafer became porous; (H) Higher magnification of the cross section of wafer five days following implantation.



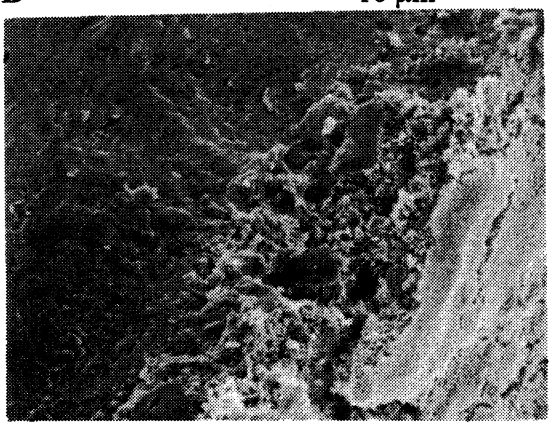
A ——10 μm



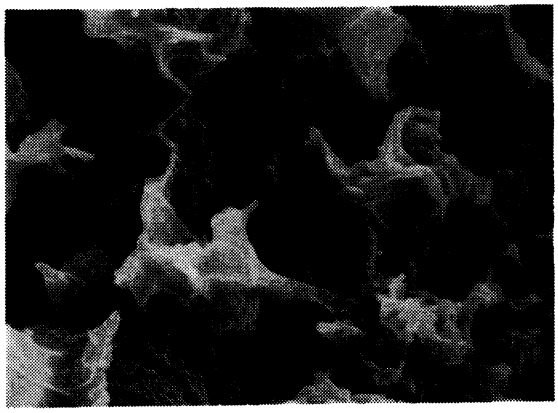
B ——10 μm



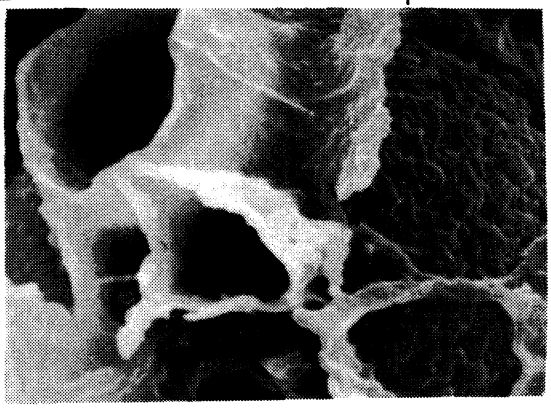
C ——10 μm



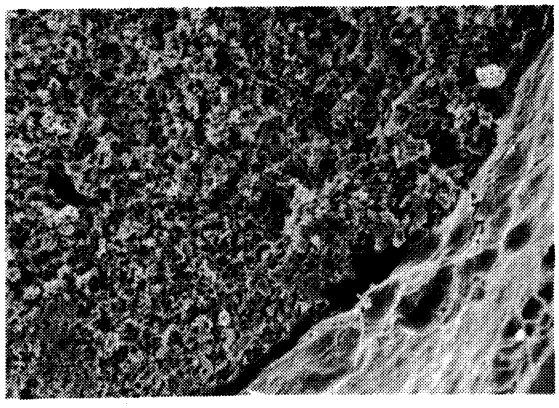
D ——100 μm



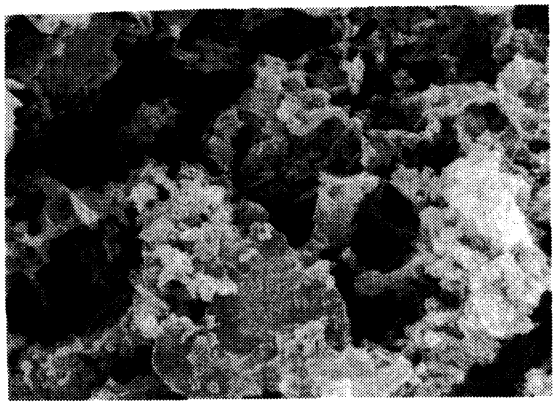
E —1 μm



F ——1 μm



G ——100 μm



H —1 μm

interior of a wafer, the structure was less and less porous (Figure 6D), demonstrating the surface erosion pattern of a polyanhydride wafer. At the porous layer (erosion zone), although some individual microspheres could still be identified, large portions of the structure were occupied by pores and connecting channels (Figure 6E&F). Higher magnification of the erosion zone (Figure 6F) revealed that the eroded microspheres had a dense structure at the external surface while the materials from the inner core had already eroded and disappeared. The remaining dense and permeable structure was probably formed by the degradation products such as CPP which need more time to dissolve and diffuse away. Five days after the wafer implantation, the entire cross section of the wafer displayed a uniformly high porosity with no individual microspheres identifiable (Figure 6G&H), indicating that water had penetrated through the whole wafer and degraded the interior as well as the exterior of the wafer. Taken together, SEM pictures of the dynamic erosion process of a BCNU-loaded polyanhydride wafer displayed the combination of both surface and bulk erosion mechanisms.

DISCUSSION

SEM has been used extensively in characterizing the internal and surface morphology of drug delivery systems and in studying their erosion and drug release mechanisms (6–9, 12). Morphological studies by SEM have revealed that the structure of polyanhydride microspheres can vary greatly depending on the technique used for preparation of microspheres and the type of drug incorporated (7–9, 12). For instance, p(CPP:SA, 50:50) microspheres prepared by a solvent evaporation technique were spherical in shape with some pores on the surface. Their internal structure was also porous due to evaporation of the volatile solvent during the drying process (9). P(CPP:SA, 20:80) microspheres prepared by a hot melt microencapsulation were also spherical with a dense and crenelated external surface (9). Polyanhydride microspheres prepared by a solvent removal technique were spherical in shape with a dense outer surface but a porous internal structure (12). Incorporation of a drug into a polyanhydride solution may further affect the morphology of the final microspheres depending on the solubility of the drug in the polymer solution. P(CPP:SA, 20:80) microspheres made by spray drying with incorporation of different drugs such as acid orange and methyl red highlighted the morphological changes caused by drug incorporation (7). Blank p(CPP:SA, 20:80) microspheres and p(CPP:SA, 20:80) loaded with 3% acid orange all exhibited a crenelated external surface (7). But when methyl red was encapsulated, the external surface of the individual microsphere was spherical in shape with few pores on the surface (7). Our study indicated that the spray dried p(CPP:SA, 20:80) microspheres were spherical with diameters ranging from 1–20 μm. Blank microspheres were smooth but porous on the external surface. Incorporation of BCNU into the polyanhydride solution changed the morphology of spray dried microspheres significantly. The microspheres became very smooth at both external and internal surfaces with no visible pores. This was very different from the morphology reported for p(CPP:SA, 20:80) loaded with 3% acid orange, probably because BCNU is soluble in methylene chloride and forms solid solutions with the p(CPP:SA, 20:80) matrix.

When a wafer was incubated in PBS, water gradually diffused into the polyanhydride matrix and degraded the anhydride bonds, resulting in the decrease in polymer molecular weight. Gaps pre-existing between packed microspheres also contributed to water penetration and subsequent degradation of the wafer. After one hour of *in vitro* incubation in PBS, the water content of the sample was approximately 2% of the dry sample weight. One day after the incubation in buffer, the water content of the sample increased to 40% of the dry sample weight. Our previous study (13) indicated that the molecular weights of the samples all decreased to less than 10 kDa after one day of incubation in PBS, regardless of the initial molecular weight of the wafers. These results are in agreement with the observations of the current SEM study. Although during the first several hours of incubation, the surface of wafers with lower initial molecular weights became more porous than that of wafers with higher initial molecular weight, very similar surface porosity was observed for both wafers after one day of incubation. It seems likely that despite the difference in the initial molecular weights, after one day of incubation the molecular weight of the insoluble copolymer portion was reduced to approximately the same low molecular weight (around 10 kDa) that would allow soluble fractions of the erosion products such as monomers and oligomers of CPP and SA to diffuse out of the matrix. With water soluble erosion products leaving the polyanhydride matrix, the weight of the eroding wafer decreased and small holes and channels were created, thus the porosity of the matrix increased sharply. SEM pictures of the eroding wafer at different time points clearly demonstrated that the morphological changes of wafer was minimum until one day of incubation in PBS. This observation also supports our previous report (13) that the weight loss of wafer and SA monomer release from the wafer was minimal during the first day of incubation, followed by a sharp increase thereafter.

Release of BCNU from the p(CPP:SA, 20:80) wafer was likely to occur via two separate mechanisms, that is, through diffusion of BCNU from the polymer matrix and through erosion of the wafer matrix as the anhydride bonds were hydrolyzed. Both mechanisms contributed to the total release of BCNU from the wafer matrix, albeit with varying proportions at different stages of the erosion. In the initial period after wafer implantation in the brains of rats (less than one day), the erosion of polymer was not apparent (Figure 5 and Figure 6C) and the bulk of the wafer was still intact. Release of BCNU during this period was likely controlled mainly by diffusion. In fact, the cumulative release of BCNU during this period was proportional to the square root of time (13), further supporting the above conclusion. As deeper layers of the wafer were being eroded by the advancing water front, the porosity of the wafer increased, resulting in increased numbers of channels and pores for water to access the interior of the wafer. The erosion process was thus extended from the exterior to the interior of the wafer. As the bulk of the wafer eroded, BCNU was released from the matrix and diffused into the interconnecting channels and pores created by polymer erosion. During these stages of wafer erosion, the release rate of BCNU was controlled by both polymer erosion and drug diffusion.

CONCLUSIONS

Using the SEM, we demonstrated for the first time that BCNU was homogeneously distributed, with no phase separa-

tion, in the spray dried p(CPP:SA, 20:80) microspheres used to fabricate GLIADEL® wafers. Morphological characterization of GLIADEL® wafers at different stages of *in vitro* and *in vivo* erosion by SEM revealed that GLIADEL® wafers underwent surface erosion in a layerwise fashion, with the porous structures gradually extending from the exterior surface into the interior of the wafer. These findings provide a direct understanding of the mechanism of GLIADEL® wafer erosion and help elucidate the mechanism of drug release from the GLIADEL® wafers.

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