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Lipids as excipient in sustained release insulin implants

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Summary

When implanted in Wistar rats with streptozotocin-induced diabetes, the release of insulin from an admixture with cholesterol which had been compressed into a pellet disc was previously found to continue for about 30 days. Several common fatty acids, their anhydrides, and glycerides were evaluated for similar suitability as a component of the implant in this study. Stearic acid and palmitic acid were found to be very promising. Especially with palmitic acid, a 25 mg piece containing 20% insulin reduced hyperglycemia to 3.7 ± 0.8 mmol/l for 43.5 ± 6.5 days when implanted subcutaneously in diabetic rats. The stable blood glucose level followed a regular food consumption pattern. But under scheduled feeding, the level varied from 2.4 ± 0.3 to 6.2 ± 0.5 mmol/l. However, hypoglycemic convulsion did not occur even after fasting for 16 h. Therefore, the rat model study indicates that palmitic acid can be a useful adjunct in sustaining the release of insulin from an implant.

Introduction

Lipid materials have been used clinically as excipients for hormones (Joseph et al., 1977) and narcotic antagonists (Sullivan et al., 1976) to achieve their slow release from subcutaneous implants. Recent studies in our laboratory show that pellet discs made by compression of an insulin and cholesterol admixture, when implanted subcutaneously, can reduce hyperglycemia in Wistar rats with streptozotocin-induced diabetes for close to 1 month (Wang, 1 987). The use of pure natural materials derived from tissue constituents in the body as a component of an implant may reduce the extent of comprehensive biocompatibility testings otherwise required. There are many lipids which can also be compressed into a coherent disc without binder and are more erodible in the body than cholesterol (Chien, 1982). These lipids may also be used as the material component of an implant for sustained delivery of insulin. This report describes the evaluation of 11 lipids or derivatives for suitability as such excipients.

Materials and Methods

Lauric acid, myristic acid, palmitic acid, stearic acid, myristic anhydride, stearic anhydride, monocaprin, trilaurin, trimyristin, tripalmitin, tristearin, bovine insulin (Zn salt, 24 U/mg) and strepto-

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zotocin were purchased from Sigma Chemicals, St Louis, MO. The Betadine solution was prepared by the Purdue Frederick Co., Toronto, Ont. The Glucometer and Dextrostix are products of Miles Laboratories, Etobicoke, Ont. The E-C Cellector Tissue Sieves were ordered from Markson Science, Phoenix, AZ, and the U.S.A. Standard Testing Sieves were bought from Gilson Co., Worthington, OH. The mechanical shaker used for mixing the pellet components was the Wig-L-Bug Amalgamator model 3110B made by Crescent Dental Mfg. Co. Lyons, IL. The 13-mm Specac Pellet Die was obtained from Analytical Accessories, Dorval, Que. The Model C hydraulic Carver Laboratory Press was purchased from Fred S. Carver, Menomonee Falls, WI. Wistar rats were bred by Jackson Laboratories, Bar Harbour, MA.

Particle size separation

Particle size of the various lipids or derivatives was analyzed using stacked sieves of different mesh size. Usually, 1 g of powder was put on the top of the stack which was followed by increasing mesh size from 10 to 200 (i.e. 2 mm to 75 μm), and was shaken vigorously for 15 min. The amount of material retained on each sieve of a mesh size was calculated as a percent of the total original sample. Initially, the particle sizes of all the lipids or the derivatives were analyzed in the form as obtained from the supplier. Then the powder, as supplied, was ground with a glass mortar and pestle for 15 min. Again, 1 g of the ground material was sieved as just described to obtain the particle size distribution. Next, the powder was combined again and ground further at increments of 15 min each time to a total of 30 min, and then a total of 45 min in some cases, followed by sieving after each additional grinding to assess the limit of pulverization.

Preparation of pellet discs

An appropriate amount of a lipid or its derivative and insulin were weighed separately before transferring into a 1.5 ml round-bottomed polyethylene vial with a screw cap. A clean stainless steel bearing ball was put atop the powder and the content of the capped vial was shaken vigorously for 15 s on the Wig-L-Bug shaker. After the bearing ball was removed, the well-mixed powder was transferred to the well of the pellet die which was then placed at the center of the platform of the hydraulic press. A moderate initial compression was applied to seal the die chamber which was then evacuated for 2.5 min before a final compression of 5 metric tons was applied for another 3 min. After the compression and vacuum were released, the die was detached from its base, and the resulting pellet was pushed out of the well by tapping the protruding plunger stem of the die. A standard size pellet disc thus made weighed about 200 mg, was 13 mm in diameter, and about 1.5 mm thick.

Diabetes induction in Wistar rats

Wistar rats (b.wt.: 320–380 g) were made diabetic by intravenous injection of streptozotocin (Ganda et al., 1976) at 75 mg/kg dissolved in 0.5 ml of saline. The rats were lightly anaesthetized by ether inhalation to facilitate the alignment of the 26-gauge needle into the base of the tail vein. The rats became diabetic in less than 24 h with blood glucose level exceeding 22 mmol/l. The diabetic condition was monitored weekly and confirmed for at least 2 weeks, before the rat was used for bioassay of the lipid implants containing insulin.

Implantation of pellet disc pieces

Under ether anaesthesia, the hair on the abdominal skin of a diabetic Wistar rat was closely shaved, and the skin was swabbed with the Betadine solution. A midline incision of 7 mm in length was made about 2 cm below the sternum with a pair of small scissors. A subcutaneous pocket at least 3 cm from the cut was made toward the flank of the animal by blunt dissection. The pellet disc piece was inserted towards the end of the pocket, and the skin wound was closed with a small Michel clip. The whole procedure required only about 3 min to complete, and the animal recovered from the anaesthesia within 1 min thereafter.

Blood glucose

Blood samples were obtained by needle puncture of the tail vein. For glucose content, the blood was smeared directly on the tip of the

Dextrostix reagent strips. After 1 min, the tip was washed with water and the blue color developed was read on the reflectance photometer which gave the result in mmol glucose/l of blood (Scheffrin et al., 1983).

Results

Particle size distribution of lipid powder

All the lipids or derivatives listed in Table 1 are crystalline solids with sharp m.p. When the material was readily available, the powder as supplied was ground several times to assess the limit of pulverization that might result in a narrow particle size distribution. For the 4 fatty acids, grinding of 15, 30 or 45 min broke up the original coarse powder to fine particles mainly (> 50%) in the range of −50 to +100 mesh size (i.e. 300–150 μm). The only exception was stearic acid which showed little decrease in particle size upon grinding. As supplied, the 2 anhydrides were large granules. Brief grinding had the effect of reducing a substantial portion (> 60%) of the particle size to a range of −60 to +100 mesh (250–150 μm). Among the 4 glycerides, grinding caused most particles to distribute within the −60 to +100 mesh size range for trilaurin, and made most of the tripalmitin powder to become very fine. There was also a distinct reduction for a large fraction

TABLE 1

Screening of lipids for suitability to deliver insulin

Expicient	Insulin content (%)	Blood glucose (mmol/l) on days						
		1	2	3	4	5	6	7
Lauric acid	10	> 22	–	> 22	–	> 22	–	> 22
	20	> 22	8.6	–	–	15.2	16.4	> 22
Myristic acid	10	3.7	–	2.2	–	4.7	–	> 22
	20	3.4	–	–	3.3	–	16.4	> 22
Palmitic acid	10	> 22	18.2	–	–	7.5	–	14.6
	20	2.8	–	–	4.0	–	5.6	7.0
	30	8.4	–	–	4.5	–	3.9	4.2
Stearic acid	10	19.1	20.0	16.4	–	19.3	17.6	20.2
	20	> 22	> 22	–	–	> 22	–	19.5
	30	11.3	2.9	–	3.5	–	2.9	2.5
Myristic anhydride	10	12.1	–	18.9	–	18.5	20.4	> 22
	20	> 22	–	–	13.3	–	> 22	> 22
	30	2.1	17.4	> 22	–	–	> 22	20.1
Stearic anhydride	10	16.1	19.8	> 22	> 22	–	21.5	> 22
	20	> 22	–	–	> 22	–	–	18.4
	30	21.8	–	17.2	–	> 22	–	18.5
Monocaprin	10	CV [a]						
Trilaurin	10	4.0	4.0	–	16.8	21.0	18.6	> 22
	20	> 22	18.5	–	5.4	14.8	–	> 22
	30	11.9	–	13.4	–	14.6	–	> 22
Trimyristin	10	20.0	18.0	–	3.0	–	> 22	18.3
	20	> 22	–	13.6	–	20.3	–	> 22
	30	11.9	13.4	–	6.1	13.6	–	18.5
Tripalmitin	10	21.0	–	16.1	16.4	–	–	> 22
	20	> 22	–	–	> 22	–	–	> 22
	30	2.8	–	3.3	–	4.4	11.1	> 22
Tristearin	10	13.0	–	21.0	–	18.1	–	> 22
	20	> 22	–	–	17.1	–	> 22	21.5
	30	> 22	–	–	> 22	–	–	> 22

[a] CV stands for hypoglycemic convulsion.

(> 50%) of the trimyristin and tristearin powder to between −60 and +200 mesh sizes. Overall, the recovery after separation was good (91 ± 10%), and a sufficient amount of all the materials in the particle size range of −60 to +200 mesh could be obtained after grinding once or twice. Initial bioassays to determine the duration of the sustained release of insulin was conducted using pieces cut from the pellet disc prepared from this fraction of the ground lipid sample having a size range of −60 to +200 mesh.

Controls and placebos

Among the 50 diabetic rats, 2 were left as untreated controls for 30 days, and their blood glucose level monitored weekly was found to be > 22 mmol/l during the entire period. They lost 64 ± 7 g of their starting weight of 335 ± 12 g before induction of hyperglycemia, and appeared emaciated. Also, the diabetic rats given an 1/4 piece (i.e. about 50 mg) of placebo pellet disc made of plain lipid as those listed in Table 1 showed similar blood glucose levels to the 2 untreated controls, indicating that the excipient materials alone had no effect in correcting hyperglycemia. When the placebo study was terminated, the implants which remained palpable could be retrieved without visible fibrous encapsulation. The monoglyceride was absorbed without a trace when examined after 7 days, but no apparent changes had occurred to the anhydrides and the triglycerides after 1 month. However, in the same length of time, the distinct edges of the pieces made of palmitic acid or stearic acid had been rounded slightly by erosion. In contrast, a blister about 1 cm in diameter developed in about 3 days at the site of the implant made of lauric acid or myristic acid. The serous fluid or abscess was drained, and only incoherent remnants of the implants were found at biopsy.

In the other set of 2 controls, each diabetic animal received an 1/40 size piece (i.e. 5 mg) of the pellet disc made of insulin alone. The implant was fatal to both animals within 4 h.

Response to insulin-containing implants

In the preliminary screening, each lipid material was tested with 10%, 20% and 30% insulin content, where applicable. For implantation, the standard size pellet disc was cut, and an 1/8 piece (i.e. 25 mg) was used each time. The bioassay for response to the insulin implant in the diabetic Wistar rat was conducted in duplicate, and the blood glucose level was determined on an alternating basis between the 2 animals at convenient intervals for a period of 7 days. When hyperglycemia persisted or recurred after a given period, the implant was removed and the animal was randomized for subsequent tests. None of these animals on re-use displayed any sign of regression of their diabetic condition or reduction of hyperglycemia without the insertion of an active implant.

As already noted with the blanks, insulin containing implants made of lauric acid or myristic acid also caused subcutaneous blisters to form in a few days. When that occurred, the blood glucose value elevated at once to > 22 mmol/l. Therefore, the response to 30% insulin content was not evaluated with these 2 fatty acids as planned. Palmitic acid implants with the 3 different insulin contents all continued to maintain a reduction in blood glucose at the end of the week (Table 1). With 10% and 20% insulin, stearic acid implants did not reduce hyperglycemia to a significant extent. But when the amount of insulin was increased to 30%, an adequate response was still continuing on day 7 as reflected by the blood glucose value of 2.5 mmol/l (Table 1; the other animal had 3.1 mmol/l on day 8). There appeared to be some insulin leaching out of the implants made of myristic and stearic anhydride, respectively, but even at 30% insulin there was very little sustained action over the 7-day period. The only monoglyceride listed in Table 1 did form a coherent pellet disc with 10% insulin; however, a piece cut therefrom disintegrated after 1 h in saline. When implanted, both diabetic test animals went into hypoglycemic convulsion in about 20 h. Attempts to save the animals were not successful, as the implant was found to be completely fragmented. The 1/8 size pieces made from the 4 triglycerides all showed some activity in reducing hyperglycemia, but none lasted beyond a week. Therefore, among the 11 lipids and derivatives evaluated, only palmitic acid and stearic acid ap-

peared to be sufficiently promising to warrant further investigation.

Stearic acid particle size

After grinding for 30 min, the stearic acid powder was sieved to separate the $-80/+200$ mesh (180 μm/75 μm) size particles which were then mixed with 30% bovine insulin and compressed into a standard size pellet disc. Similarly, another pellet disc of the same insulin content was made from stearic acid powder in the $-40/+50$ mesh (425 μm/300 μm) range. A piece, each about 1/8 of the size of the whole pellet disc, was inserted subcutaneously into several diabetic Wistar rats. The insert made from coarse stearic acid lowered the blood glucose to 3.5 ± 1 mmol/l in all 4 test animals for 15–24 days. Similar reduction in hyperglycemia was observed in all 5 diabetic rats with the implant made of the fine particle mixture and for the same range in days. When the ground, but unsieved powder was used to make the pellet disc and similar portions thereof were implanted, the 3 diabetic rats showed steady weight gains with reduction of hyperglycemia again in the range of 3.5 ± 1 mmol/l, and for 16–24 days (Fig. 1). More of the major $-40/+50$ mesh fraction was then isolated from the unground powder and put in the small-ball mill. After shaking for 15 s as in the mixing with insulin, the milled powder was sieved to obtain the particle size distribution and was found to be essentially identical to a sample after 15 min grinding by hand. Therefore the use of the vibratory ball mill to achieve good mixing with insulin has effectively reduced a coarse powder into a common distribution of particle sizes. Henceforth, no attempt was made to select a particular range of particle size for the preparation of pellet discs.

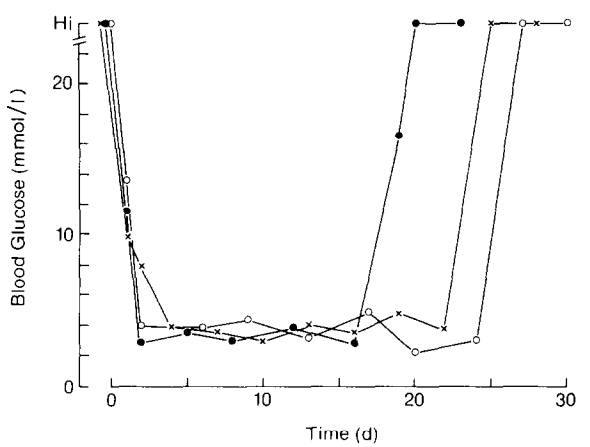

Fig. 1. Reduction of hyperglycemia in 3 diabetic Wistar rats, each by an 1/8 size piece of pellet disc made of stearic acid containing 30% bovine insulin. Normoglycemia is 5.5 ± 0.5 mmol/l. Exceeding 22 mmol/l, a "Hi" indication is shown on the reflectance photometer.

Enhancement of action by further subdivision

Pieces of the pellet disc made of stearic acid with 20% insulin content that were found to be inactive in the preliminary screening (Table 1) were cut into chips of about 1 mm^3 in size with a utility knife. An amount of 25 mg which was equivalent in weight to 1/8 of the standard size pellet was implanted subcutaneously. The subdivision was effective in causing the glucose level to fall from > 22 mmol/l to 4.3 ± 2.1 mmol/l for 20–29 days in all 4 diabetic rats.

Palmitic acid as excipient

The 2 diabetic rats, each having an 1/8 size piece of the pellet disc made of palmitic acid and 20% insulin in the preliminary screening (see Table 1), were kept beyond the 7-day period, and their blood glucose was monitored twice every week. In addition, 2 more diabetic animals were implanted with an 1/8 size piece each, which was cut from a different pellet of the same insulin content. Fig. 2 shows the blood glucose level during the 37–50 day service-life (Wang, 1987) of the 4 implants which was by far the longest observed for any of the lipids tested. The other 2 animals with the 30% insulin implant in the preliminary screening study (see Table 1) were also kept beyond the 7-day period. However, both died of hypoglycemia on day 16 and day 25, respectively. Another 2 animals were used to test if the service-life may be lengthened by doubling the size of the implant. But the 1/4 size piece containing 20% insulin proved to be fatal to both animals within 2 weeks. As in the case of the stearic acid just described, subdivision of the marginally active pellet disc containing 10% insulin (see Table 1) into small chips did result in better reduction of

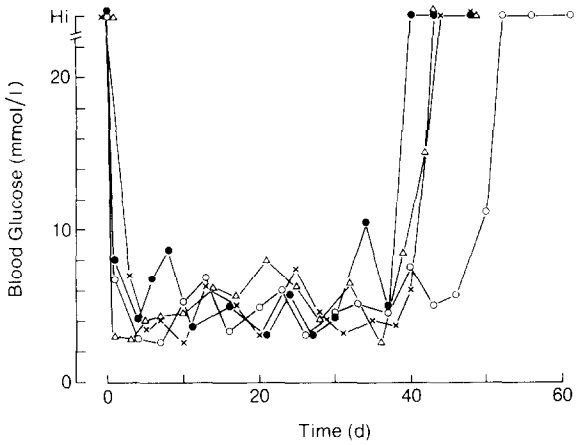

Fig. 2. The reduction of hyperglycemia in 3 diabetic Wistar rats by 1/8 size piece made of palmitic acid containing 20% insulin. The blood glucose of the test animals shown was closer to the level of normoglycemia at 5.5 ± 0.5 mmol/l, and the service-life was more reproducible at about 40 days in comparison to Fig. 1. One animal was used twice (●——● and ○——○) on 2 separate occasions in this experiment.

hyperglycemia when tested in 3 diabetic rats. But there was much larger fluctuations in the blood glucose level (Fig. 3) as compared to the data shown in Fig. 2, and the service-life was also variable. Therefore, an 1/8 size piece containing 20% insulin appears to be an optimal combination.

Feeding and blood glucose change

Since the implant cannot vary with changes in insulin demand, 3 diabetic rats with active implant of 20% insulin in ground (for 15 min), but unsieved, palmitic acid powder were monitored for food intake and blood glucose level. In a 22-day period, their blood glucose remained at 3.7 ± 0.8 mmol/l, and the food consumptions were also very consistent. When monitored weekly from 17.00 to 09.00 h the next day with lights off, each ate 20.7 ± 0.3 g. However, in the following 8 h with lights on, the nocturnal animals ate 14 ± 0.5 g each even with brief intervals when no food was eaten at all. On 2 other occasions, the feeding was scheduled between 09.00 and 17.00 h by allowing the animals to eat at will for 1 h in the morning. Thereafter, the food was withheld for about 4 h until afternoon, when it was restored again for 1 h. Fluctuations became more apparent, and their post-prandial blood glucose level rose to 6.2 ± 0.5 mmol/l which decreased in about 4 h to 2.4 ± 0.3 mmol/l, but the withdrawal of food was well tolerated. There were 2 other separate occasions when food was withheld overnight for 16 h, and the blood glucose of the test animals dropped to about 2.0 mmol/l both times, but still no hypoglycemic convulsion occurred.

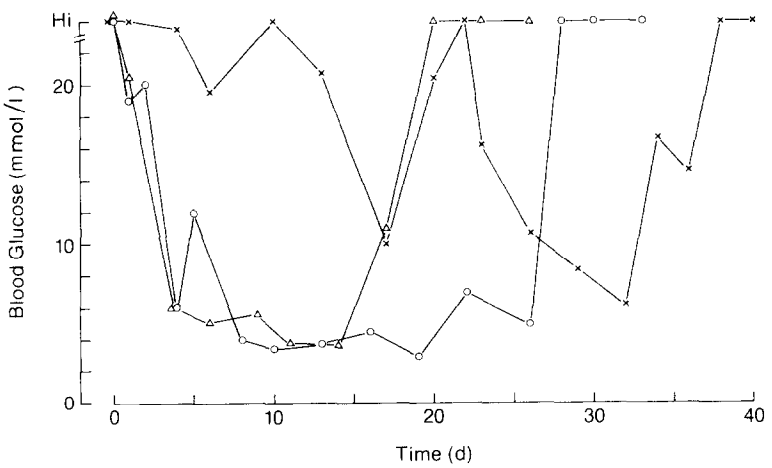

Fig. 3. Three Wistar rats each implanted with 25 mg in weight of chips cut from the marginally active pellet discs made from palmitic acid containing 10% insulin. The response was enhanced, but there were considerable variations in blood glucose level and the implant service-life.

Discussion

The longest acting insulin preparation for injection lasts for 36 h, but its action peaks only from 18 to 24 h. A steady basal level of insulin can be better maintained by continuous infusion with a pump, but insulin aggregation from solution remains a serious problem. In another approach, non-degradable polymer implants with entrapped insulin powder have been developed which can reduce hyperglycemia continuously in diabetic rats (Brown et al., 1986). These small implants contain sufficient insulin to last for many months, and can be modulated externally (Edelman et al., 1985) to compensate for post-prandial demand. Efforts are continuing to synthesize various biodegradable polymers (Mathiowitz et al., 1987) to eliminate the need for explanting the polymer device after the insulin supply is exhausted.

The testing of cholesterol as a material component for insulin implant (Wang, 1987) is the result of a study conducted in this laboratory (Wang, 1986, 1988) which indicates that the use of a synthetic polymer component is not mandatory. In the present work, 11 readily available lipids and their derivatives were screened for suitability as excipients (Table 1). The maximum insulin content evaluated has been set at 30%, because insulin is quite expensive, and higher amounts may shorten the service-life (Wang, 1987). Among the 4 triglycerides, trilaurin, trimyristin and tripalmitin appeared somewhat promising at 30% insulin content (Table 1). The monoglyceride tested as well as lauric and myristic acids eroded too fast and were not suitable at all. The most promising lipids are the 2 long-chain fatty acids. At 20% insulin, a 25-mg piece cut from the pellet disc made of palmitic acid was able to keep the blood glucose near the normal level beyond the 7-day period when most others ceased to function. To achieve the same result with stearic acid, 30% insulin was required (Table 1).

Aside from the appropriate number of carbon atoms in the *n*-alkyl chain, it is not immediately apparent with respect to the particular property possessed by these 2 fatty acids which contributes to the sustained release of insulin observed. The melting points of these 2 acids are close to tripalmitin and tristearin, but the triglycerides only sustained the insulin release briefly (Table 1). Also, all the lipid powder used to make the pellet disc for bioassay includes a representative distribution of particle sizes (i.e. -60 to $+200$ mesh) which did not provide much insight to explain the extended release of insulin dispersed in palmitic and stearic acids. In addition, the vibratory ball milling would have turned a powder into a common range of particle size distribution anyway as observed with stearic acid. However, cutting the pellet disc made of stearic acid into chips of about 1 mm^3 in size did result in the sustained reduction of hyperglycemia for almost 1 month with just 20% insulin content. Therefore, the sustained activity observed for these chips may be partly due to the increased exposure of the channels at the interior through cutting. Since erosion also takes place to some extent, it appears that the actual reasons for the unique property of stearic and palmitic acids as excipients are quite complex and cannot be attributed to physical factors alone.

The implant made with a lipid excipient cannot respond to varying insulin demands; therefore it is important to know the extent of blood glucose fluctuation that might result in dangerous convulsions due to feeding schedules. The blood glucose level of the diabetic Wistar rats with an implant was found to remain fairly stable under self-feeding or scheduled feeding conditions. Even under extreme circumstances when food was withheld for 16 h overnight, the animals tolerated the fasting well without hypoglycemic convulsion. Therefore, the insulin-containing implant made of palmitic acid is safe and adequate in the treatment of streptozotocin-induced diabetes in rats.

The present report has demonstrated that the potency of insulin administered subcutaneously can be preserved by compression with a lipid excipient which also sustains the insulin release with continuous reduction of hyperglycemia for many weeks in diabetic rats. Since biotechnologically produced insulin is now available, and the lipids are natural products present in the body, toxic, teratogenic or immunogenic effects should not be expected of such implants. Another important aspect is that the subdivision of the pellet disc pieces further into small chips did not result

in sudden insulin overdose, and therefore accidental fracture of a large piece of the implant in vivo is not expected to pose any danger to the recipient. Thus, formulations (Chien, 1982) or instruments may be developed to facilitate the insertion of the small chips by injection instead of minor surgery to implant the larger pieces through skin incision.

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