BIODEGRADABLE, THERMOSENSITIVE IMPLANT FOR APPROXIMATING CYLINDRICAL STRUCTURES: A PRELIMINARY STUDY

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A new invention, i.e., a biodegradable, thermosensitive hybrid gel composite in the form of a thin sheet, was used as an implant inside the rat and guinea pig to test its tissue reactions, degradation, and function as a bridging agent. Tissue tractions in subcutaneous tissue, in muscle, around a peripheral nerve, and around an artery were mild. Degradation made rapid progress, starting on the third day and completed in 2–3 weeks. The hybrid gel, when wrapped around the cut ends of a peripheral nerve or an artery, functioned well as a bridging tube. In the case of the cut nerve, regenerating fascicles crept through the tube which protected them from fibrosis. In the case of the cut artery, patency of flow was maintained, and arterial wall healing was complete in 2–3 weeks. The hybrid gel composite contracted at body temperature, thus holding well to body tissues. Its biodegradable and inert nature offers the potential for future use as a tissue wrap and bridging agent.

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Tubular structures such as tendons, nerves, and vessels can be approximated by suturing. Alternately, tubular implants are used to bridge such structures in an attempt to save time and with special indications: for instance, tubular stents have been used to connect vessels, and silicone tubes have been used to bridge cut peripheral nerves.[1–3]

Although the purpose of using bridging implants is to save time and make the surgery more precise, those who have applied the implant realize that, unless they are dealing with a large structure, it might be technically difficult to fulfill the need. Putting the tubular implant around the tissues to be approximated might not be difficult, but to prevent slippage, it is often necessary to put in a couple of stitches, which might not be as easy as one would expect. This explains why such bridging implants are not always popular.[4,5]

If the implant possesses the ability to grip the tissues so that stitching or other means to hold it becomes unnecessary, it might have more potential uses.

MATERIALS AND METHODS

One of us (C.W.) invented a novel hybrid gel by embedding billions of thermally sensitive spherical microgels (~400 nm) into a protein gel network. It can undergo fast shrinking at a rate of 0.6–1.0 mm/min at 37°C.

The Hybrid Gel Composite

The hybrid gel composite was prepared by a special polymerization process already documented.[1] Spherical poly(N-isopropylacrylamide) (PNIPAM) microgels were embedded inside a gelatin gel network. The spherical microgels have a diameter of 0.3–1.0 μm in the swollen state; they were embedded into a gelatin gel network. The shrinking of the microgels could lead to a fast shrinking of the gelatin gel. The embedded microgels were attached to the gelatin gel network by physical adhesion, and not by chemical bonding. Therefore, it was expected that the shrunken microgels could slowly detach themselves from the gelatin gel network and be absorbed by normal tissue mechanisms, because the shrunken microgels have a

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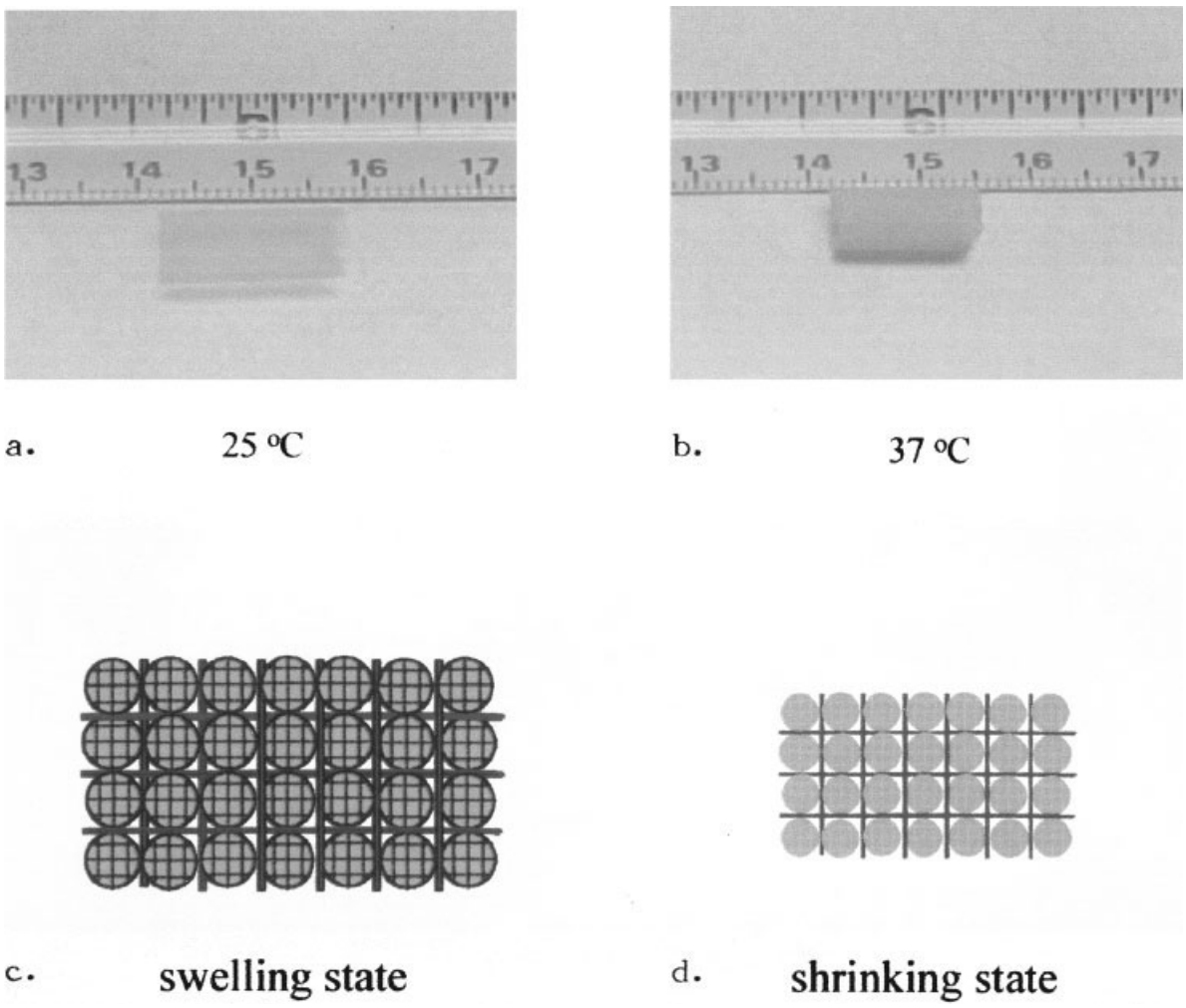

Figure 1. ***a:*** *Thermosensitive polymer at 25°C.* ***b:*** *Same polymer at 37°C.* ***c:*** *Polymer at 25°C.* ***d:*** *Same polymer at 37°C.*

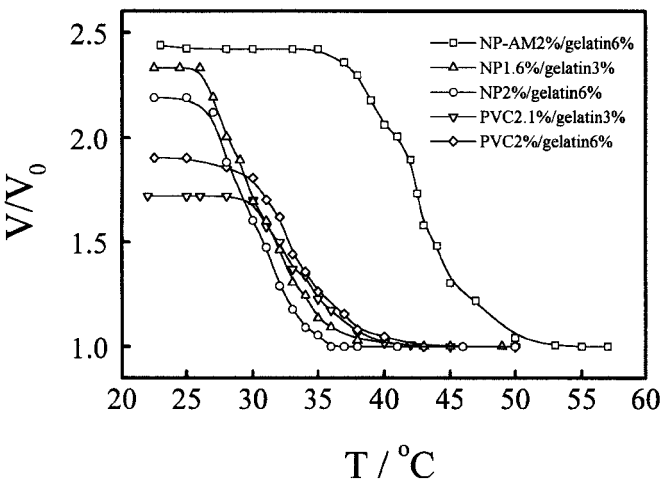

Figure 2. Behaviors of different models of thermosensitive polymers at different temperatures.

Table 1. Tissues Chosen for Studies of Reactions to Polymer Implant*

	Rat	Guinea pig
Subcutaneous	Yes	Yes
Artery	Yes	No
Peripheral nerve	Yes	No
Tendon	Yes	No
Muscle	Yes	Yes

**Five animals were used for each dedicated period of study, i.e., days 3, 10, 20, and 90, for a total of 20. On designated date of sacrifice, only 3 animals were studied. The extra numbers were required to make allowances for animal mortality. In total, 15 rats and 10 guinea pigs died before study of the implantation area.*

diameter of only 0.1−0.3 μm. Spherical PNIPAM microgels were synthesized by emulsion polymerization. Then, 3.84 g of N-isopropylacylamide (NIPAM), 0.0636 g sodium dodecyl sulfate (SDS), and 0.0735 g of N,N-methylenebis(acrylamide) (MBAA) were dissolved in 240 ml of deionized water. After bubbling nitrogen

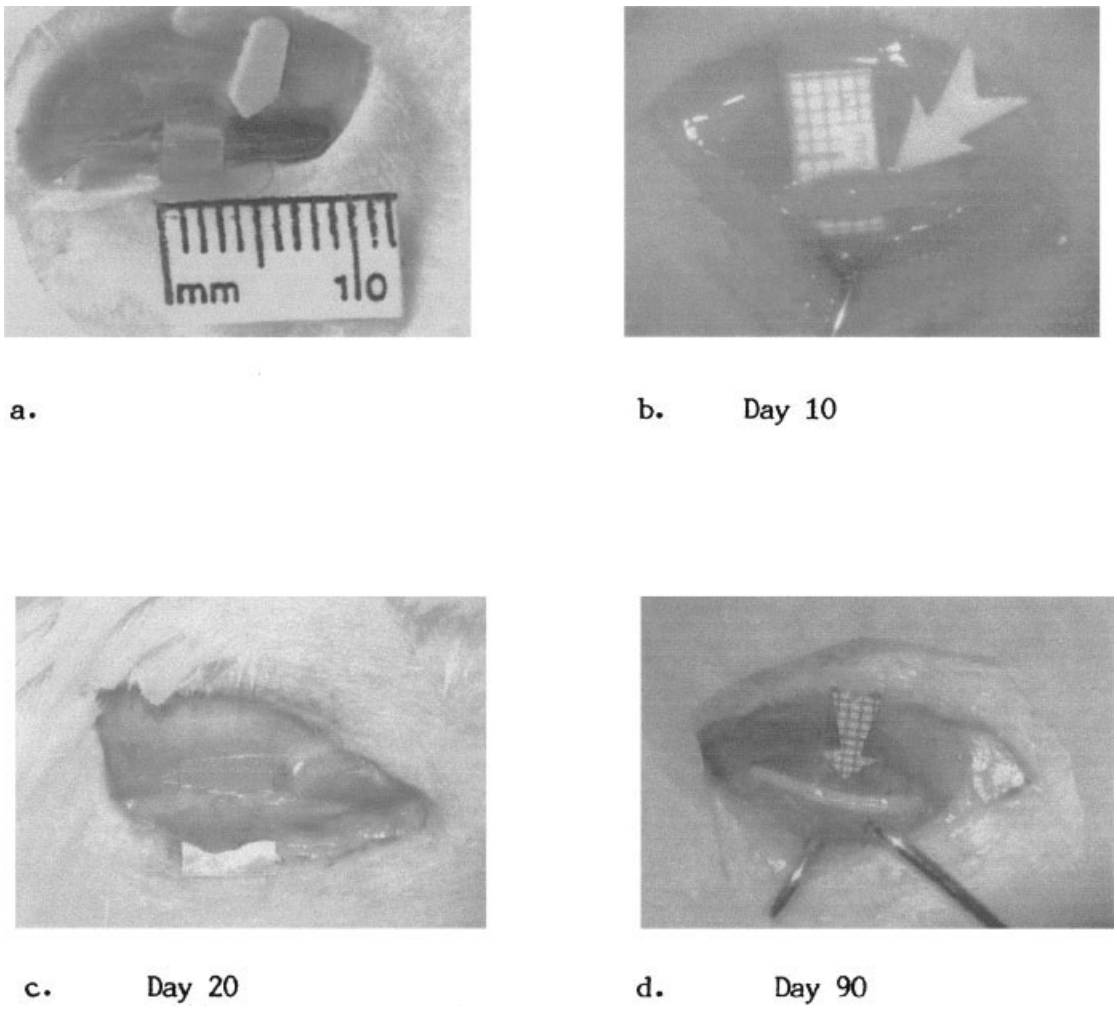

Figure 3. ***a:*** *Sciatic nerve of rat, bisected across, and cut ends wrapped with thermosensitive gel.* ***b:*** *Day 10.* ***c:*** *Day 20.* ***d:*** *Day 90.*

treatment for 30 min at 70°C, 0.1737 g KPS in 25 ml deionized water was added to the solution. Polymerization was carried out at 70°C for 5 h. The PNIPAM microgel was purified and concentrated by repeated centrifugation and washing at a higher temperature (35−37°C) with deionized water. The composite was prepared by adding a proper amount of purified PNIPAM microgels (2−4 wt%) into a gelatin solution (2−6 wt%) at ~30°C with stirring. The mixture was quickly injected into a model with the desired shape, such as a tube or a disk, and placed inside a refrigerator. The resultant gel was immersed in 0.5% glutaric dialdehyde solution for 1 day to chemically cross-link the gelatin molecules. The unreacted reactant was removed by washing the gel with deionized water.

The gel is naturally sticky and attaches to soft tissues by physical adhesion. When body heat is transmitted from the soft tissues to the gel which is thermosensitive, it shrinks, thus gripping the surrounding tissues even more firmly.

When the polymer gel was made in the laboratory, different compositions could be tried: we prepared the gel composites with different ratios of gelatin and polymer. The total concentration of gelatin ranged from 1−6%, and the polymer gel concentration was in the range of 1−3%. Mechanical and biological testing had been done to eliminate unsuitable composites, and this report concentrates on the model poly(N-isopropylacrylamide) microgel (NP-MG) 1.6% and gel 3%, which was found most appropriate for biological implantation.[6] We measured the gel strength. When the gelatin content is lower than 1%, the gel composites do not have sufficient mechanical strength. As for biological testing, we found that some of the poly(N-vinylcaprolactam) microgels with a lower content of gelatin have serious tissue reactions.

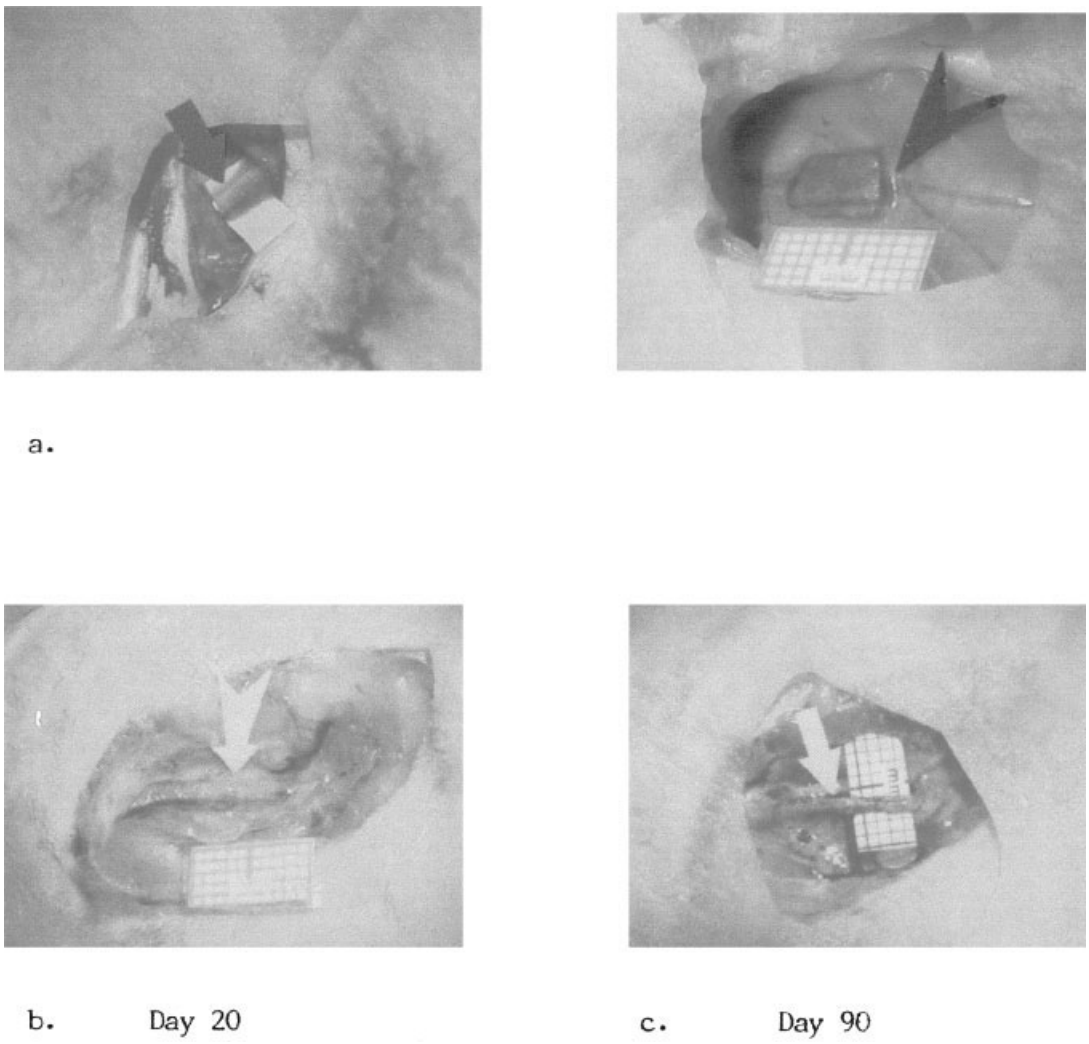

Figure 4. ***a:*** *Femoral artery of rat, bisected across, cut ends wrapped with thermosensitive gel.* ***b:*** *Day 20.* ***c:*** *Day 90.*

Use of the Thermosensitive Gel in Tissue Repair

The product used in this report was a polymer in the form of a soft gel consisting of thermosensitive microspheres which were laid on a gelatin network to form a thin sheet (Figs. 1 and 2).

This thermal-sensitive sheet of polymer gel could be wrapped or passed around the ends of cut tubular structures, e.g., cut nerve or vessel, in vivo, so as to maintain continuity of the cut ends.[7,8] Because of the higher temperature of the soft tissues in contact with the gel, shrinkage occurred. The shrinking structure therefore tightened around the cut ends to be bridged, and stitching could be avoided.

The Experiments

Testing body reactions to the polymer at different tissue sites. The in vivo biological responses to the polymer gel had to be tested carefully before it could be accepted as a surgical implant. Different tissues that were likely to be put in contact with the implant when surgically used were chosen. Different tissues might have different responses to the polymer, and how the polymer would degrade with time also needed to be clarified.

The tissues chosen included the following: subcutaneous tissues, nerve, artery, tendon, and muscle (Table 1). The animals used included adult male Wistar rats and guinea pigs. Short-term and medium-term observations were made when the implant sites were inspected after periods of 3, 10, 20, and 90 days.

Testing the repair and regeneration of cut nerves and arteries. The polymer gel-gelatin sheet was used to wrap the cut ends of the sciatic nerve of the rat, so as to form a bridging tube. The rats were euthanized after 3,

Table 2. Tissue Reactions to Thermosensitive Polymer Gel Implant

		Day 3	Day 10	Day 20	Day 90
Subcutaneous tissues	Acute inflammation	Severe	Mild Hyperemia +	No more hyperemia	Nil
	Chronic inflammation	Nil	Some fibrocytes	Little fibrosis	Nil
	Polymer gel degradation	Nil	Fragmentation	Macrophages + Only fragments seen	Very few fragments
Arterial wall	Acute inflammation	Mild hyperemia	Nil	Nil	Nil
	Chronic inflammation	Some fibrocytes	Continuity Little fibrosis	Continuity Little fibrosis	Continuity
	Polymer gel degradation	Nil	Fragments seen	Macrophages + Some fragments	All disappeared
Sciatic nerve	Acute inflammation	Severe	Hyperemia +	Continuity obvious	Nil
	Chronic inflammation	Nil	Fibrocytes	Fibrosis (mild)	Fibrosis (mild)
	Polymer gel degradation	Nil	Fragmentations	Macrophages + some fragments seen	No implant visible
Tendon tissues	Acute inflammation	Severe	Hyperemia +	Mild hyperemia	Nil
	Chronic inflammation	Nil	Fibrocytes	Little fibrosis	Little fibrosis
	Polymer gel degradation	Nil	Fragmentation	Fragmentation Macrophages +	Fragments still seen Macrophages +
Muscle tissues	Acute inflammation	Severe	Hyperemia ++	Hyperemia +	Nil
	Chronic inflammation	Nil	Fibrocytes	Fibrocytes	Nil
	Polymer gel degradation	Nil	Macrophages + Only fragments	Macrophages ++ Very few fragments	No implant visible

10, 20, and 90 days to retrieve the repaired nerve for gross and histological study (Fig. 3).

The femoral artery of the rat was used for the study of arterial repair. After cutting through, the arterial ends were prevented from separating by putting two 10 ''0'' stitches to sling the edges together while leaving gaps of about 1 mm open. Then the approximated site was wrapped around with a thin sheet of thermosensitive polymer gel. The rats were euthanized on days 3, 10, 20, and 90 for the inspection and retrieval of the bridged artery (Fig. 4).

Only rat models were used in the artery and nerve studies. Control groups of equal numbers of animals were supplied for both nerve and artery studies. In control groups, the conventional stitching method using 10 ''0'' stitches was done for the repair.

RESULTS

The gel material was known to be nontoxic. Both rats and guinea pigs survived well after the gel implantations. When the implantation sites were inspected, there were neither gross nor histological suggestion of toxicity.

Tissue Reactions

The tissues around the implanted polymer gel were inspected for inflammatory changes and at the same time the degradation of the gel material was recorded. A summary is given in Table 2.

Five rats and guinea pigs were used for each group of subcutaneous and intramuscular implants. Tissue reactions in the rat were not different from those in the guinea pigs. Only the rat model was used for the artery and nerve studies.

The overall tissue reactions were mild in the early acute inflammatory stage. Of the four types of tissues, muscle showed the most severe acute inflammatory reactions. Hyperemia subsided quickly in 20 days, when degradation of the polymer became obvious. Chronic inflammatory reactions and fibrosis were also mild when the implantation reached 90 days. The expected foreign-body reaction with plenty of fibrotic tissues was certainly absent in all tissues tested. Even the most reactive tissue, i.e., muscle, only showed slightly more accumulation of macrophages, but not much fibrosis.

The tissue reactions around the implanted polymer were studied grossly as well as histologically. Similarly, the regenerative behavior of arterial and nerve tissues was analyzed microscopically. Histological sections were made of tissues retrieved from the implantation sites at 20 and 90 days for analysis of tissue behavior in response to implant degradation.

On day 20, fragments of foreign bodies were seen encircled by macrophages. Lymphocytes were scarce. Inflammatory infiltration was minimal. Specimens of different tissues were quite similar with the exception of muscle tissues, which showed more inflammatory infil-

Table 3. Nerve Regeneration

		Day 3		Day 10		Day 20		Day 90	
		Implant group	Stitched group	Implant group	Stitched group	Implant group	Stitched group	Implant group	Stitched group
Gross appearance	1. Regeneration of Schwann cells and nerve fibrils	Early	Not so obvious	Obvious	Early	Complete connection	Complete connection	Intact nerve	Intact nerve
	2. Fibrosis	No	No	No	Mild	No	Mild	No	Minimal
	3. Integrity of implant	Intact	–	Early degradation	–	Degraded	–	Disappeared	–
Histological section	Average number of fascicular bundles at cross section at cut gap	Nil	Nil	2	2	5	5	7	7

tration at an early stage and more macrophages from day 10 onwards. Gel degradation was much quicker in the intramuscular implants.

On day 90, gel fragments appeared scarce, and so did macrophages. Only a few lymphocytes were seen, and some macrophages appeared necrotic. It was obvious that gel absorption started after 1 week and became more or less complete after around 90 days.

Nerve Regeneration

The cut sciatic nerve, separated from the surrounding tissues with the polymer-gel wrap, was allowed to regenerate within the protective tube. Three rats were used for each group of experiments and controls. The results are shown in Table 3.

Arterial Wrap

Three rats were used in each group of patency experiments and controls, and the patency of anastomosed femoral arteries using the polymer gel wraps compared with stitching is described in Table 4.

Examination of the implant sites included a careful search for the implanted polymer. Early cracking was observed from day 10, after which fragmentation became obvious on day 20. No more polymer gel was visible 3 months after the implantation.

DISCUSSION

The thermosensitive polymer-gel sheet used was the product of multiple early tests, using polymers of different protein concentrations and different morphological forms, which included preformed tubes and fluid forms. Tubes were difficult to manage surgically, while the fluid form was messy. The sheet form appeared a good compromise. Since the gel contracted on contact with live tissues (which possessed a higher temperature to initiate the contracture), it easily maintained its tubed form when it was wrapped around cylindrical structures such as arteries, nerves, or tendons.[9]

The unique property of the polymer gel was therefore thoroughly utilized to give extra binding power when it was wrapped around the cylindrical biological structures. Our experiments on arteries and peripheral nerves showed that wrapping did facilitate the maintenance of arterial patency and allowed natural nerve regeneration, while the foreign-body reaction to the implanted polymer was mild.

A second property of the polymer gel was its degradability. If an approximating implant gradually degraded and the degradation gave minimal tissue re-

Table 4. Implant group vs. Stitched Group

	Day 3		Day 10		Day 20		Day 90	
State of flow	Implant group	Stitched group	Implant group	Stitched group	Implant group	Stitched group	Implant group	Stitched group
Patient flow	2	2	2	3	3	2	3	3
Blocked	1	1	1	–	–	1	–	–
Degradation of polymer-gel gelatin tube wrap	Day 3		Day 10		Day 20		Day 90	
Sheet intact	3/3		–		–		–	
Early cracking	–		3/3		–		–	
Early fragmentation	–		–		3/3		–	
Grossly invisible	–		–		–		3/3	

actions, it might provide extra benefit as an implant, because foreign-body reaction is temporary, and moreover, removal would become totally unnecessary. Our results on tissue reaction and implant degradation were most impressive in that acute inflammatory changes and foreign-body reactions appeared minimal, fibrosis was mild, and implant degradation was grossly complete after 90 days of implantation.

While more improvement could be looked forward to in terms of a further strengthening of the polymer-gel gelatin to make it easier to handle, early human use could be planned for vascular and nerve approximations.

Two more potential uses of this polymer could be considered for muscle and tendon repair. When muscle tissues were disrupted, repair by ordinary suturing gave very poor results because of extensive fibrosis around the repaired site and replacement of the damaged muscle with fibrous tissues. These events led to a gross loss of muscle function. If a tissue wrap could be applied to the damaged site to bring about approximation while minimizing fibrosis, the functional results might be better.

Tendon repair for disruptions was also adversely affected by adhesions which did not allow functional gliding. A biodegradable interposition wrap that induced the minimal fibrosis would be a most useful consideration.

While we are excited to see these preliminary promising results of the thermosensitive polymer gel, we are engaging in trials to detect its possible potential as a bone glue or if the polymer could be strengthened.

The polymer proved to be harmless in experiments on the rat and guinea pig, and may therefore be safely studied further.

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