Design and Manufacture of a Large-Scale Collagen Protein Model for Educational Use

by

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Submitted to the Department of Mechanical Engineering
in Partial Fulfillment of the
Requirements for the Degree of

Bachelor of Science

at the

Massachusetts Institute of Technology

June 2004

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ABSTRACT

A meter-scale model of the fibrous 1CGD molecule, a synthetic collagen-like peptide, was modeled and manufactured for educational use. We chose to model and manufacture the 1CGD molecule because collagen is of biological importance, the molecular coordinates are well-established, and because the molecular structure and geometry of 1CGD are representative of many general concepts important to the study of proteins.

The 1CGD molecule consists of three polypeptide chains. Our model is three separately manufactured chains intertwined with one another after manufacture. The A chain of the 1CGD molecule was first modeled using the SolidWorks computer aided drawing (CAD) software. The CAD model was then converted to a file that enabled us to manufacture a master part via a three-dimensional printing (3DP) process available from Z Corporation. The 3DP master part was used to manufacture a room temperature vulcanized (RTV) brush-on blanket mold and rigid plaster mother mold. The mold, created by artist Bob Shure at Skylight Studios, was then used to cast parts using a two component liquid plastic that cures to a solid material. The parting lines of the final parts were cleaned up and the final parts painted to complete the model.

This thesis details the specifics of the manufacturing requirements, constraints, attempts, and ultimate process used to make a functional 1CGD model.

Thesis Supervisor: David Gossard
Title: Professor of Mechanical Engineering

ACKNOWLEDGEMENTS

Many thanks go out to Professor David Gossard and Dick Fenner for their help and guidance over the course of this project. And thanks to fellow students, Heather Doering and Jane Yoon, for their help during the initial stages of this project. Thanks also to fellow student, Andy Kutas, for his expertise in computer modeling.

Special thanks to artist Bob Shure of Skylight Studios in Woburn, Massachusetts. Without the expertise of Mr. Shure, we never would have been able to manufacture such a high quality mold. His insight and help were invaluable in the timely completion of this thesis.

I would also like to thank my friend, Joe Stark, for helping me cast parts out in the cold at odd hours of the night. Without his help maneuvering the mold and tightening the compression straps, my parts would have continued to be of severely poor quality.

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Chapter 1

INTRODUCTION

1.1 Motivation

The molecular structure and geometry of complex molecules, such as proteins, are often difficult to visualize and understand from a one-dimensional representation of the molecule on paper. Many of the key concepts and structural aspects of the molecules are difficult to represent and often lost in one-dimensional representations of the molecule. For example, the secondary, tertiary, and quaternary structures of proteins are often difficult concepts to visualize without a three-dimensional representation of the protein, thus students learning about these concepts often find them esoteric. So, in order to facilitate the understanding of some of the structural and geometric aspects of complex molecules, it would be beneficial to have a tangible three-dimensional model of a representative molecule to illustrate the key concepts.

There are currently computer models and programs available that allow a rendition of a molecule to be virtually manipulated in such a way as to give the appearance of three-dimensionality. But these renditions have their limitations as well. Some truly three-dimensional models of complex molecules exist and are often used in classrooms to demonstrate molecular concepts. However, many of these models use a representation called the ball-and-stick model which is an abstraction of the actual structure and geometry of molecule. In the abstraction of the actual three-dimensional structure and geometry, some important information is often lost. The students are still required to visualize the geometry of the molecule to some extent, and still must sort through the often confusing and jumbled connections between the "balls" and "sticks."

Few three-dimensional models exist to show the actual structure and geometry as determined through a variety of molecular mapping techniques. These models are called space-filled models and show how the electron shells overlap by representing the molecules as interlocking spheres instead of individual spheres connected by rods as in the ball-and-stick model. Both the space-filled and ball-and-stick models of molecules are important representations and students in high school and college biology and chemistry classes are introduced to both representations. The space-filled model can help give students a better understanding for concepts like secondary, tertiary, and quaternary structures of proteins.

A model which combines the two three-dimensional representations, the ball-and-stick and space-filled models, into one model would be ideal. Such a model would allow students to integrate their understanding of each representation and thus help them to apply their understanding of the differences and limitations of each representation to further work with either representation. A combined model would likely take the form of a color coded ball-and-stick model embedded inside a clear plastic space-filled model. Technology for creating such a model does currently exist, however, this thesis will focus on the design and manufacture of the space-filled model. The addition of an embedded ball-and-stick model is out of the scope of this thesis and is left for future investigation.

Thus, the ultimate goal of this thesis work is to produce a space-filled model of a representative protein to help students understand the three-dimensional structure and geometry of a complex molecule. A collagen protein was chosen as a representative molecule because it illustrates many of the common concepts and structural aspects of protein molecules while still being a relatively simple protein molecule.

1.2 Scope and Organization

This thesis will examine the design and manufacturing processes available, and the processes ultimately used, to create a meter-scale space-filled model of a collagen protein for low volume production. The importance and relevance of the collagen protein will be explained and justified. The computer modeling process will be discussed briefly, but this thesis will concentrate on examining the techniques available for mold and master part production, as well as casting material selection in the context of the complex geometry of a collagen protein. The challenges and trade-offs encountered during the manufacturing process will be discussed and analyzed, and suggestions for future improvements and further investigation will be addressed.

Chapter 2

COLLAGEN PROTEIN MOLECULE

2.1 Biological Importance of the Collagen Protein Molecule

Proteins are important biological compounds that often have very complex structures and geometries. Collagen is a protein that is found throughout the human body. “In fact, it is the most abundant protein in the body (representing 25 percent of the total body protein) [1].” It is especially prevalent in the extracellular matrix where it provides structure and support to cells [1]. Collagen can be found in tissues such as skin, tendons, ligaments, and the eye [1]. Collagen would be a good protein for students to study because of its prevalence and its importance in the human body. Aside from its biological importance, collagen protein is an ideal protein to endeavor to model and manufacture, because aspects of its structure and geometry illustrate important concepts and aspects of many proteins.

2.2 Structure and Computer Solid Model of Collagen Protein

For the purposes of this project, the protein designated as 1CGD, a synthetic, collagen-like peptide, will be used as our biological molecule. 1CGD’s molecular coordinates are fairly well established and documented in the RCSB Protein Databank http://www.rcsb.org/pdb/index.html. The molecular coordinates for naturally occurring biological collagen are not currently well established, and thus the synthetic collagen will be used.

1CGD, like a naturally occurring collagen protein molecule, is a fibrous protein consisting of three chains wrapped around one another. Along each chain is a repeating pattern of residues. Each chain has a total of 30 residues in a repeating pattern of proline, hydroxyproline and glycine. In the center of each chain is an alanine residue. Figure 1 shows a 1CGD protein molecule. Two chains are solid colors, while the third chain has been colored by residue.

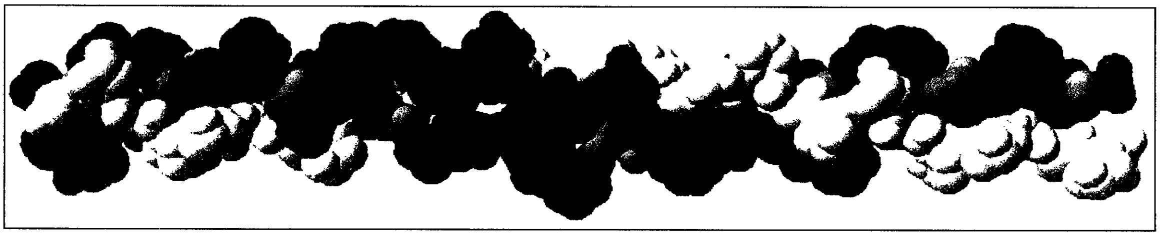

Figure 1: Graphical Representation of the Space-Filled Model of the Synthetic Collagen-Like Peptide 1CGD. In this representation, two chains (the yellow and green chains) are solid colors. The third chain is colored by residue. Red represents proline, blue represents hydroxyproline, orange represents glycine, and black represents alanine.

The molecular coordinates and atomic structure of each chain are available from the RCSB Protein Database in downloadable format. The data obtained from the RCSB Protein Database gives all the necessary information to create a solid model of the space-filled representation.

A colleague, Andy Kutas, used the information from the RCSB Protein Database to create a solid model of the 1CGD protein's A chain. The solid model was then converted into an STL file, which mathematically slices the computer model into thin slices so that a rapid prototyping machine can build the part. The STL file was then passed onto Z Corporation so that they could make a master part of the 1CGD protein's A chain. Master part manufacturing techniques are discussed in more detail in section 3.1.

Chapter 3

MOLDING AND CASTING TECHNIQUES

There is a plethora of techniques available to manufacture molds and to cast parts. Many of these molding and casting techniques have been available for centuries, while others have become available as manufacturing processes and materials have improved and new technologies have been developed.

It is important to select a process appropriate for the production of the part of interest. Factors such as cost, production time, production volume, tolerances and quality, material requirements, part strength requirements, and part geometry place limitations on the types of manufacturing processes one should consider. Oftentimes, engineers will design parts for manufacturability and/or assimilability; that is engineers will design parts with a specific manufacturing process and/or assembly process in mind so that the part meets all the necessary requirements, but is also optimized for efficient, economical, and relatively simple production given a certain process.

However, we do not have the luxury of designing a collagen protein molecule for manufacture or assembly. Nature has determined the structure and geometry of our part. We may be able to determine or control the other design variables and select a manufacturing process that will allow us to meet our requirements, but we are bound to the geometry. The geometry of a collagen protein, it turns out, severely limits the types of manufacturing processes we can consider. Our options are limited because of the complex, rounded geometry of the protein and the absence of a mathematically perfect parting line or lines. Common processes such as injection molding and other molding and casting processes that require a simple parting line simply will not suffice here because there is no line or lines of symmetry that would allow a part to be released from a mold cavity. Thus, a less traditional, more artful, molding and casting process must be used.

Several processes currently exist for molding and casting geometrically complicated parts. Three-dimensional printing (3DP) and room temperature vulcanization (RTV) technologies are two such manufacturing processes used to create complex parts. Three-dimensional printing was chosen as the method for creating a master pattern and a permanent shell and model, and an RTV process was used to create the mold with which to cast multiple parts. The justification for choosing these technologies and their advantages and disadvantages are discussed in the following sections.

3.1 Creating the Master Pattern: Rapid Prototyping Techniques

In order to create a mold with which to cast parts, a master pattern or part must first be manufactured. A variety of different manufacturing methods and processes exist to produce master patterns or prototypes, however, many processes are not appropriate for manufacturing a protein molecule because of the complex, rounded geometry and the absence of a mathematically perfect parting line or lines. The complex geometry makes the part difficult to machine on conventional machine tools such as mills and lathes. The absence of a mathematically perfect parting line or lines makes processes such as injection molding infeasible. Luckily, rapid prototyping technologies exist. Rapid prototyping processes allow a part to be "printed" from a computer model by building up material in thin layers.

Several rapid prototyping processes are currently available. Rapid prototyping processes include stereolithography (SLA), selective laser sintering (SLS), and three-dimensional printing (3DP), and are discussed in sections 3.1.1, 3.1.2 and 3.1.3 respectively.

3.1.1 Stereolithography

American scientist Charles Hull developed the first rapid prototyping technology, stereolithography (SLA), in 1982 [2]. Although the SLA process has been improved over the last twenty-two years since its conception, its basic principle remains the same and the process is a leading technology for rapid prototyping. The basic principle behind the

SLA process is photo-polymerization, whereby a liquid photopolymer solidifies when exposed to ultraviolet light [2].

First, a CAD model of the desired part is created and converted into STL format. The STL format slices the part into appropriately thick layers so that, when the information from the CAD model is fed into the SLA machine, a laser can selectively expose appropriate material to ultraviolet light in thin layers [2, 3]. The part is thus built up in thin layers until the part is completed. A schematic of the SLA process is shown in Figure 2.

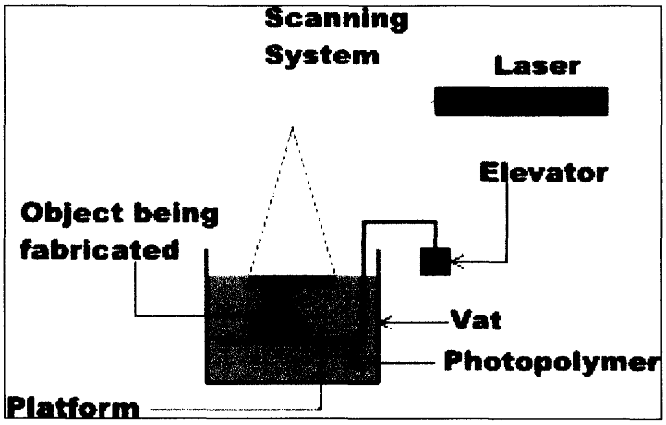

Figure 2: SLA Process Schematic [3]. The SLA rapid prototyping process uses a laser to selectively harden areas of a photopolymer to create a part.

The SLA process has advantages and disadvantages. The process is useful for prototyping because it is a relatively quick and cost-effective method for producing small numbers of complex parts [2]. The time required to create a part is largely determined by the volume of the part and not by its complexity, because it is not necessary to manufacture any complicated tooling [2]. However, the photopolymer materials used to create the parts in an SLA process do not generally have desirable mechanical properties [2]. The parts are generally not strong enough for use in a real product. A much greater variety of mechanical properties can be achieved by using the SLA part as a master part and then creating a mold so that parts can be cast out of a more suitable material [2]. Not only are the parts created via SLA often not strong enough to be integrated into the final product, but some parts with large, heavy overhangs may need temporary support structures built up along with the part so that the features are supported while the part is being formed [4]. Furthermore, the SLA process inherently causes up to 5% shrinkage [2] depending upon the geometry and volume of the part. Such shrinkage could present a problem if tight tolerances on the prototype are required. Another disadvantage, especially for our purposes, is the restriction of the color of the parts produced by the SLA process. SLA parts must be painted or otherwise finished if color is desired on the completed part.

Ultimately, the SLA process was not selected for creating the master pattern of the collagen protein molecule. Initially, our goal was to create a color-coded ball-and-stick model of the collagen protein and embed it within a clear space-filled model. If a process that allowed the user to select different colors and integrate color into the part creation on a material level instead of on a post-processing level (i.e. painting), the production of the ball-and-stick model would be simplified. While the integration of color into the space-filled model was not critical, it would have been easier to select a process and company that could produce both parts for us using the same process. So even though we were compelled to reconsider the scope of this thesis and eliminate the ball-and-stick model integration with the space-filled model, the process ultimately chosen to produce the space-filled model was acceptable and well motivated at the time. Thus, while the SLA process would have been a perfectly usable process for our current purposes it was not ideal given our initial motivation, since several better processes are available that allow the integration of color. The three-dimensional printing process ultimately selected and used to create the master model is discussed later in section 3.1.3.

3.1.2 Selective Laser Sintering

The selective laser sintering (SLS) process was developed by the American company 3d Systems after the SLA process was developed [5]. The process uses a carbon dioxide laser to fuse together, or sinter, powdered material into solid three-dimensional objects [6, 7]. The SLS process allows a greater variety of materials to be used to make the part. Plastics, plasters, and metal can all be used in the SLS process [6, 7].

Similar to the SLA process, first a CAD model is converted into an STL file which mathematically “slices” the part into thin layers to be sintered by the laser. In order to make the part, a thin layer of the powdered material is moved from the powder supply cylinder into the fabrication cylinder. The laser then outlines the first layer of the part, sintering the material together. The fabrication piston lowers and the process repeats until the part is fully formed [6, 7]. A schematic showing the SLS process mechanism is shown Figure 3.

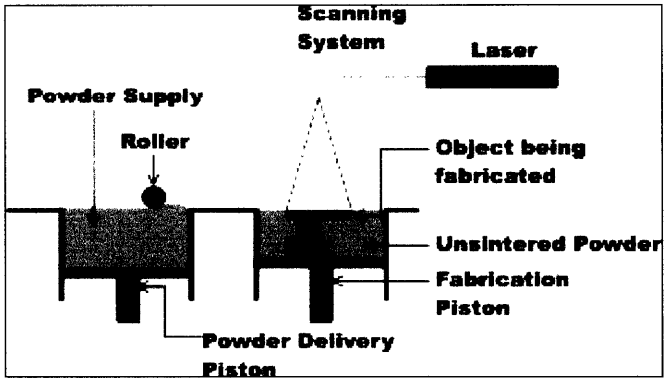

Figure 3: SLS Process Schematic [7]. The SLS rapid prototyping process uses a laser to fuse together powdered material into a solid, three-dimensional part.

The SLS process is much more versatile than the SLA process, but limitation still exist. Oftentimes, the process is more expensive due to special laser requirements or post-processing if materials other than plastic are used. Similar to the SLA process, any parts made via SLS would require the addition of color later as pigmentation is not integrated with the material sintering. Thus, parts are the color of the material powder and/or binding compounds used in sintering some ceramics and metals.

Ultimately, the SLS process was not chosen for our application, largely for the same reasons the SLA process was not chosen. The SLS process would have been perfectly suitable, just as the SLA process would have been, but given our initial scope of the project, we chose a process that would allow us to integrate color in our model. The process chosen for the manufacture of the master pattern, 3DP, is discussed in the next section.

3.1.3 Three-dimensional Printing

Three-dimensional printing (3DP) is a relatively new technology developed at the Massachusetts Institute of Technology after the SLA and SLS processes [6, 8]. The process is similar to the SLS process in that material is built up out of a powder base. However, in the 3DP process a binding agent is deposited into the power bed to fuse together thin layers of the powdered material instead of sintering the material with a laser as in the SLS process [6, 8]. The 3DP process uses an ink-jet printer head to build up thin layers from an STL file and a piston to lower the part as the layers are bound together [6, 8]. A schematic of the 3DP process is shown in Figure 4.

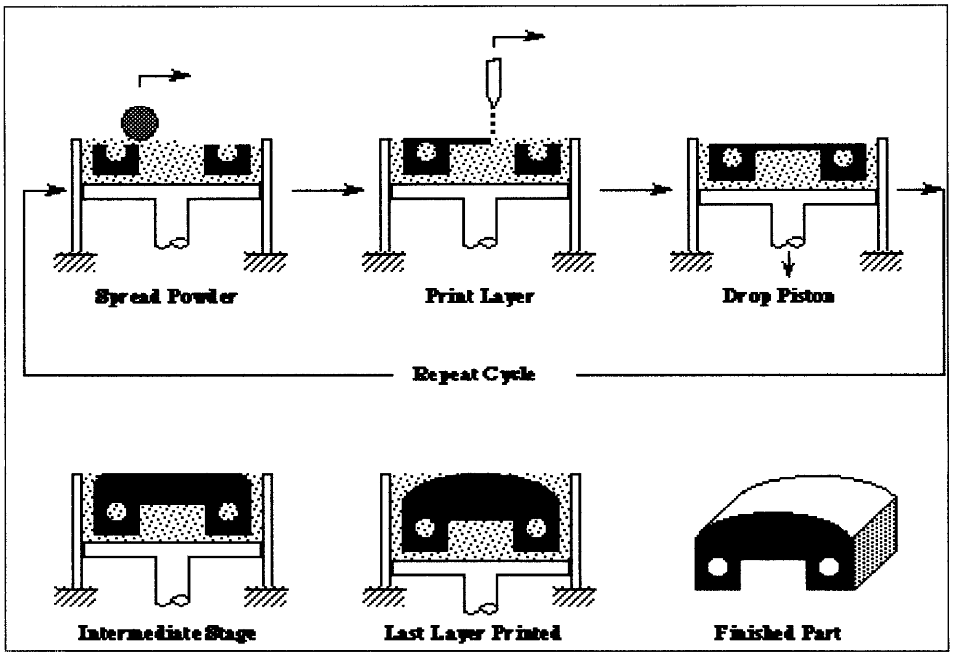

Figure 4: 3DP Process Schematic [6, 8]. The 3DP rapid prototyping process uses an ink-jet like head to deposit a binding agent in a powder bed of selected material to build up a finished three-dimensional part.

Depending upon the desired material behavior, some post processing may be necessary. Sometimes additional coatings of binding agent are applied to the part to reinforce the binding agent deposited in the building process to make the part stronger, while other times the part must be manipulated to break some of the powdered material free from the binding agent lattice to achieve rubber-like material properties. A wide variety of materials can be used in this process. Metals, ceramics, plastics, and many other materials that can be reduced to a powder consistency are all suitable for this process. Similarly, several different binding agents are available and selection of an appropriate binder/part material combination is largely based on the desired properties of the material of the finished part.

The 3DP process has several key advantages over the SLA and SLS processes that we considered for the manufacturing process of the master part. The most immediate reason we chose the 3DP process was the ability for the addition of multiple colors to be integrated in the part production, which would eliminate a large degree of post processing. A leading 3DP company, Z Corporation, has developed this color printing system [9]. With our initial focus on printing color coded ball-and-stick models as well as the master part of the space-filled model, the ability to eliminate post processing, e.g. painting, would have been extremely helpful. Furthermore, because of Z Corporation's connections to MIT and its local facilities, it made working with them a bit more convenient and less expensive.

Thus, the master space-filled model was created using the 3DP process at Z Corporation. From the materials Z Corporation's processes offered, the materials selected were a starch-based powder and a super-glue-like binding agent. David Tedder at Z Corporation made these material selection suggestions to us based on our desired material properties and the intended use of the part. The finished master part is shown in Figures 5 and 6.

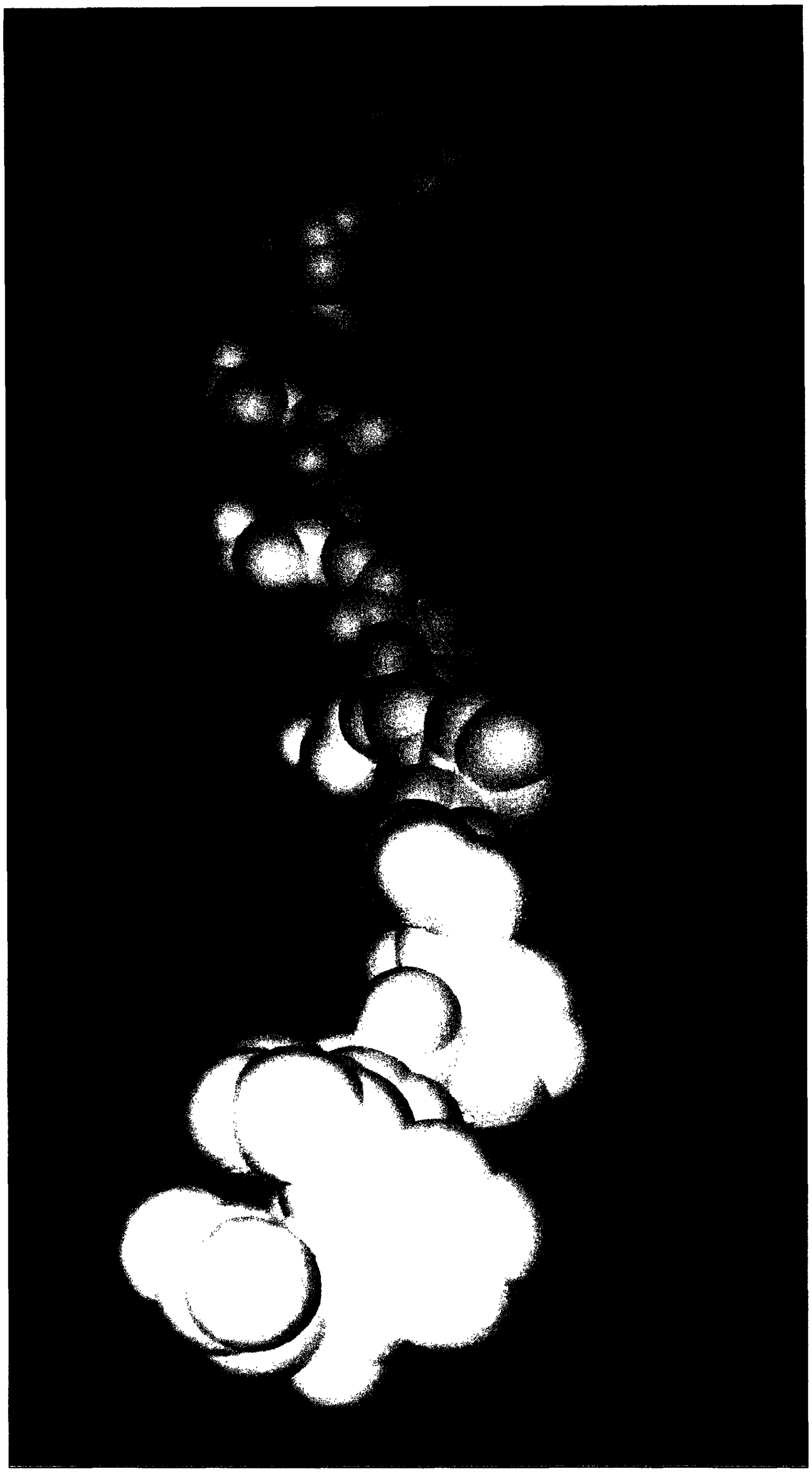

Figure 5: Finished Master Part. The finished master was manufactured via a 3DP process at Z Corporation. The master part is made out of a starch-based powder compound and a super-glue-like binding agent. This part was used to create the mold from which parts will be cast.

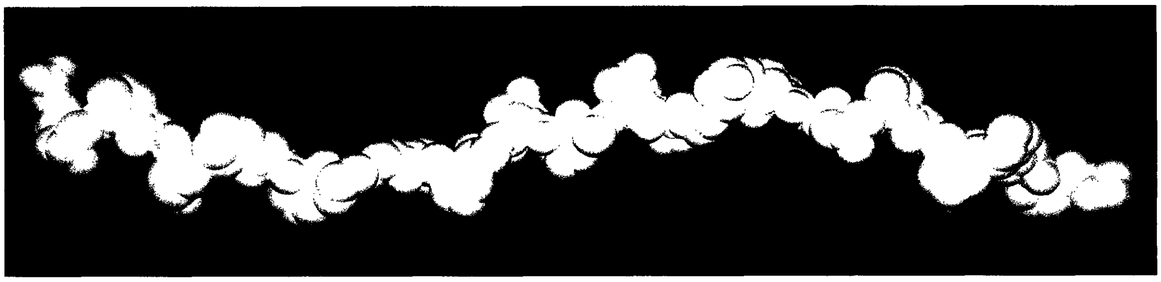

Figure 6: Finished Master Part. The A chain of the collagen protein molecule model is shown here. This model was used as the master part for the creation of the mold from which castings would be made.

The master part was used for the mold creation, discussed in section 3.2.

3.2 Room Temperature Vulcanization (RTV) Mold Making Techniques

Room temperature vulcanization (RTV) is a mold making process that uses rubber-like molding material that cures at room temperature to form a flexible, robust mold. The RTV mold is ideal for parts that have complicated geometries that do not have "perfect" parting lines that allow the master part to be easily released from a rigid mold. Because the RTV mold is flexible, it allows parts to be easily removed by mold deformation without sacrificing the mold. While RTV molds are extremely useful for complex part geometries, these molds cannot withstand high volume production volumes without being replaced. But, given our complex part and low production volume, the RTV mold is an ideal choice for our molding technique.

There are multiple methods of making molds from RTV materials and a variety of different RTV mold materials to choose from. The primary RTV methods considered for the mold making process for our purposes are discussed in sections 3.2.1 and 3.2.2. The RTV mold material selection process and ultimate choice are discussed in section 4.1.

3.2.1 Poured Molds

Poured molds can range from quite simple to very complex depending upon the master part. Poured molds can be as little as one-piece or as multi-pieced as necessary [10]. A one-piece poured mold is perhaps the easiest and simplest mold to make [10]. To make a one-piece poured mold a mold box is constructed and the master part positioned inside the mold box. Molding material is poured around the part in the mold box, allowed to cure and the master part is then removed, leaving a cavity in the mold for casting parts. A schematic of the one-piece mold is shown in Figure 7.

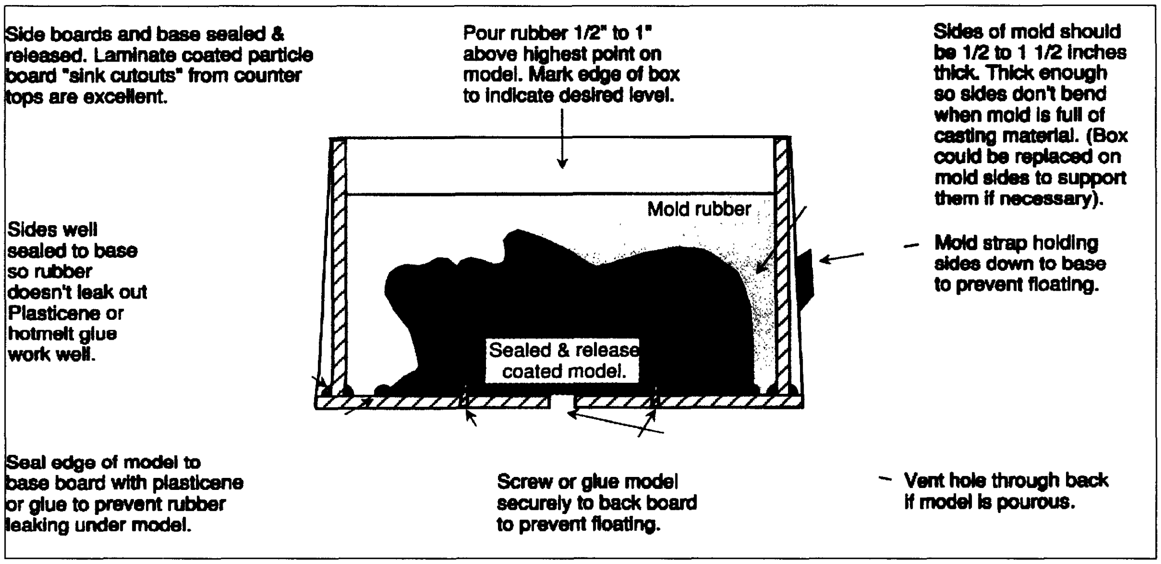

Figure 7: One Piece Poured Mold [10]. The simplest mold is the one-piece poured mold. Basic instructions and a schematic explain how to make a one-piece poured mold.

More complex molds can be made by using the same pouring technique, by cutting the mold into multiple parts as necessary in order to release more complicated master parts from the molding material. The mold can then be reconstructed within the mold box for casting. A schematic of a more complicated mold and simple instructions for how to create it are shown in Figure 8.

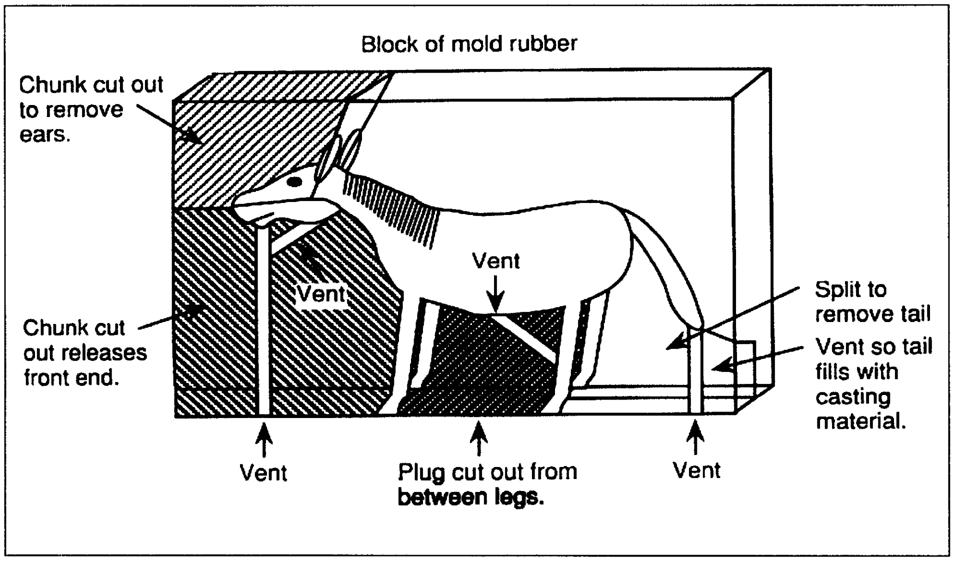

Figure 8: Multi-part Poured Mold [10]. Molds for more complicated parts can be created by cutting a poured mold into multiple parts and putting it back together within a mold box for casting parts.

Initially, we intended to use this multi-part poured mold technique to create our own mold here at MIT. The bench-level experiment to determine the feasibility of using this method, given our part's complex rounded geometry and the resources, skill level, and time available, is discussed late in section 5.1.1.

Parts with an easily defined parting line can be molded in two separate halves and the halves assembled into a two-part poured mold [10]. Ultimately, a modified two-part poured mold technique was used to create the final mold. Because of the necessary level of skill and experience to create a good, working, two-part mold with a complex parting line, we ultimately outsourced our mold production to an artist at Skylight Studios. A more detailed discussion of the creation of the final mold is presented later in section 5.2.2.

With any mold making technique, gates and vents must be located appropriately so that casting material can be poured into the mold and air can escape. Without proper gates and vents, the cast part may have incomplete features or air pockets in areas where air could not escape the mold. Gates and vents should be incorporated into the master part or can be created by removing the mold material where necessary. Depending upon the mold material and the source of the master part, one gate and vent location method may be preferable to the other.

3.2.2 Brush-On Blanket Molds

Another technique for creating molds for complex parts is the brush-on blanket mold making technique. The brush-on blanket mold technique requires that the RTV mold material be brushed onto the master part and a mother mold used to provide rigid structural support while casting. The technique can be done with translucent RTV mold making material and the parting line created much as it would be for the creation of a multi-part poured mold, or a process similar to the one typically used for traditional two-part molds can be used.

For the purposes of our application, the following method for making a brush-on blanket mold was used. First, a clay bed was created and the master part embedded within the clay such that an appropriate parting surface was created. Registration marks were created on the parting surface and then the RTV mold making material was brushed over the exposed half of the master part. The RTV mold making material was allowed to cure and the clay was then removed from the other half of the master part. The newly exposed half of the master part was then covered in RTV mold making material and allowed to cure. The two halves were then separated and the mother shells made around them. Plaster was used to create the mother shell. The RTV mold halves were then placed inside the plaster mother mold and the mold was ready for use.

3.3 Casting Techniques

Casting, while it may seem simple and straight forward, takes a significant level of skill and practice to produce quality parts. Manufacturing may be a science, but there is also a degree of art in it as well, especially when it comes to molding and casting complex parts. Choosing a casting material and pouring techniques are perhaps the most important aspects of learning to cast quality parts. The experiments and knowledge gleaned from experienced molding and casting artisans will be discussed in section 5 and the casting material selection process will be discussed in section 4.2.

Chapter 4

MATERIALS SELECTION

Material selection is an important component to the design and manufacturing process. Materials must be selected to meet certain requirements so that the finished parts perform as expected and required. The material selection process is intimately related to the manufacturing processes available and must be considered in the design of the part(s) and the selection of the manufacturing process.

For our purposes, it was important to select appropriate and compatible materials for the mold and the castings. The mold and finished cast parts each had requirements that had to be considered when selecting appropriate materials. The material selection process for the mold and castings are discussed in sections 4.1 and 4.2 respectively.

4.1 Mold Material Selection

Selecting a material for the mold is important because the mold material will determine how easily cast parts can be removed from the mold (e.g. is mold release necessary?), how the mold maintains itself after multiple production runs, and how the part is geometrically released from the mold (i.e. is the mold flexible enough to allow easy removal of complex parts?).

Initially, when the plan of action was to create a multi-part poured mold by creating the necessary parting line by eye as we cut out the master part, we established several requirements for selecting a mold material. For our application, the mold material must be:

- Flexible enough to easily release the complicated parts

- Not require mold release (so parts could be painted if desired) when used with our choice of casting material
- Able to withstand a production life of at least 20 parts
- Reasonable pour and demold times (should be able to take time pouring and demold within 36 hours)
- Reasonable viscosity (should be relatively easy to pour)
- Compatible with suitable casting material(s)
- Translucent to allow a parting line to be created by eye as the master part is being cut out of the mold material.

These requirements guided our mold material selection in the initial stages of our mold making experiments. Based on these requirements, the RTV mold making material called PlatSil® 71-40, available from Polytek Development Corp., was chosen [10]. "The PlatSil® 71 Series RTV Silicone Rubbers are two-component, additional-cure, platinum-catalyzed, high tear strength, flexible mold compounds [10]." Table 1 lists some important properties of the PlatSil® 71-40 material.

Table 1: Relevant Physical Properties of PlatSil® 71-40 RTV Mold Making Material [10]. The following physical properties are important factors to consider when selecting a mold material. PlatSil® 71-40 met all of our requirements and was thus chosen as the mold making material for the multi-part poured mold attempt.

Physical Property	**PlatSil® 71-40 RTV Mold Material**
Mix Ratio, by weight	5B to 1A
Hardness, Shore A	40
Pot Life, minimum	60 min
Demold Time @ 25°C (77°F)	24 hr
Color	Hazy Transparent
Viscosity, mixed	25,000 cps
Cubic inches/pound	25
Specific Gravity	1.1
Shrinkage during cure	Nil
Mold release required?	No if casting with polyester, epoxy, polyurethane resins, waxes and many other materials

Ultimately, based on the results of a bench-level experiment with making a mold of a smaller similar part to our master part, it was determined that the multi-part poured mold technique would not be ideal for our purposes. Detailed discussion of the results of our experiments and the reasons why the multi-part poured mold technique was not ideal are presented in section 5.1.1.

As already discussed, the decision was then made to have a skilled molding and casting artisan create a two-part brushed-on blanket mold encased in a rigid shell for us. With this new technique came new requirements for the mold material. Now the mold material need not satisfy the last color requirement which greatly increases the number of options available. Given our requirements and the intended use of our mold, artist Bob Shure at Skylight Studios determined the best mold material for our use. He used Mold Max 30, available from Smooth-On Plastics. Mold Max 30 is a silicone RTV mold material compatible with wax, urethane, polyester, gypsum, low-melt metals, and epoxy casting materials [11]. The relevant material properties of Mold Max 30 are listed in Table 2.

Table 2: Relevant Physical Properties of Mold Max 30 RTV Mold Making Material [11]. The following physical properties are important factors to consider when selecting a mold material. Mold Max 30 met all of our requirements and was recommended by artist Bob Shure. It was chosen as the mold making material for the final mold.

Physical Property	**Mold Max 30 RTV Mold Material**
Mix Ratio, by weight	100A to 10B
Hardness, Shore A	30
Pot Life, minimum	45 min
Demold Time @ 25°C (77°F)	24 hr
Color	Light Blue
Viscosity, mixed	25,000 cps
Specific Gravity	1.18
Specific Volume	23.5
Shrinkage during cure	0.004 in/in
Mold release required?	No if casting with polyester, epoxy, urethane resins, waxes and many other materials

4.2 Casting Material Selection

Molding and casting are intimately related, and so too are the material selections for the two processes. Some casting materials cannot be used in certain types of molds. The two materials must be compatible if the molding and casting process is to be successful. So when materials selection for the molding compound began, so too did the selection of the casting material. Like the evolution of the material selection process for the mold making compound, the material selection for the casting compound also evolved as our vision and goals for the project changed.

Initially, when we were operating under the assumption we would create our own multi-part poured mold and cast a clear space-filled model with an embedded ball-and-stick model of the collagen protein, a certain casting material was chosen to meet our requirements. Initially, we required that the casting material be:

- Transparent color
- Compatible with a suitable mold material
- Able to accept an embedded object
- Reasonable cure time (cures within 36 hours).

Given these requirements a water-clear polyester resin material manufactured by Environmental Tech called Castin' Craft Clear Casting Resin was selected. Other similar materials such as Smooth-On Plastic's Clear Cast product would also have worked just as well, but Castin' Craft Clear Casting Resin was available at local craft stores and thus easier to obtain on short notice.

Experiments were conducted with the Castin' Craft Clear Casting Resin, as discussed later in section 5.1.2, and the material was deemed suitable for the initial purposes, but when the scope of the project changed, so too did the casting material. With the elimination of the embedded ball-and-stick model within a space-filled model, the requirements for a clear plastic casting material and the ability to embed another part inside the casting material were no longer necessary and the options for a casting material were greatly increased.

At the suggestion of the artist at Skylight Studios who made our final mold, a urethane based casting resin was chosen for the final parts. A product called Smooth-Cast 320, manufactured by Smooth-On Plastics, was ultimately chosen. Smooth-Cast 320 is ideal for our purposes because of the fast cure time, the easy mixing ratio and the water-like viscosity all of which make the plastic easy to work with. The relevant material properties of Smooth-Cast 320 are outlined in Table 3.

Table 3: Relevant Physical Properties of Smooth-Cast 320 Casting Resin [12]. The following physical properties are important factors to consider when selecting a casting material. Smooth-Cast 320 met all of our requirements and was suggested as a good material choice by a skilled molding and casting artisan and was thus chosen as the casting material for the final parts.

Physical Property	**Smooth-Cast 320 Casting Resin**
Mix Ratio, by volume	1A to 1B
Ultimate Shore Hardness	70D
Pot Life, minimum	3 min
Demold Time @ 25°C (77°F)	7-10 min
Color	Off-white
Viscosity, mixed	80 cps
Specific Volume	26.6 in^3/lb
Specific Gravity	1.05 g/cc mixed
Shrinkage during cure in./in.	0.01
Tensile Strength	3000 psi

Now that the materials selection process for the molding and casting processes has been discussed, the details of our experiments, and ultimate molding and casting process will be discussed in Chapter 5.

Chapter 5

EXPERIMENTAL PROCEDURES AND RESULTS

A good deal of experimentation and hands-on learning occurred while working to manufacture a functional model of a collagen protein. Chapter 5 details the process, steps, and techniques tried and eventually used to create the mold and the castings of the collagen protein molecule. This chapter should provide ample documentation of the work already completed and provide guidance to any investigator who may wish to continue work on the project in the future.

5.1 Mold Construction

The first major experimental task was to determine what type of mold making technique would work best for our purposes. After ample research and consideration of the different techniques discussed in Chapter 3, we decided to first try to use the multi-part poured mold technique to manufacture our own mold at the facilities at MIT.

5.1.1 Poured Mold

In theory, the multi-part mold making process involves pouring translucent RTV mold material over a master part, letting the mold material cure and then cutting out the master part with a knife to create a multi-part mold that can then be used to cast parts. The process sounds pretty straight-forward, however, when attempted practically, it did not work as well as we had expected.

In order to test the multi-part poured mold technique, we first constructed a small representative model of the collagen protein chain to use as our master part. The representative model, while not the same structure or geometry of the actual collagen protein chain master part, did have the same rounded, twisted geometric characteristics. Figure 9 shows the master part we made for the experiment.

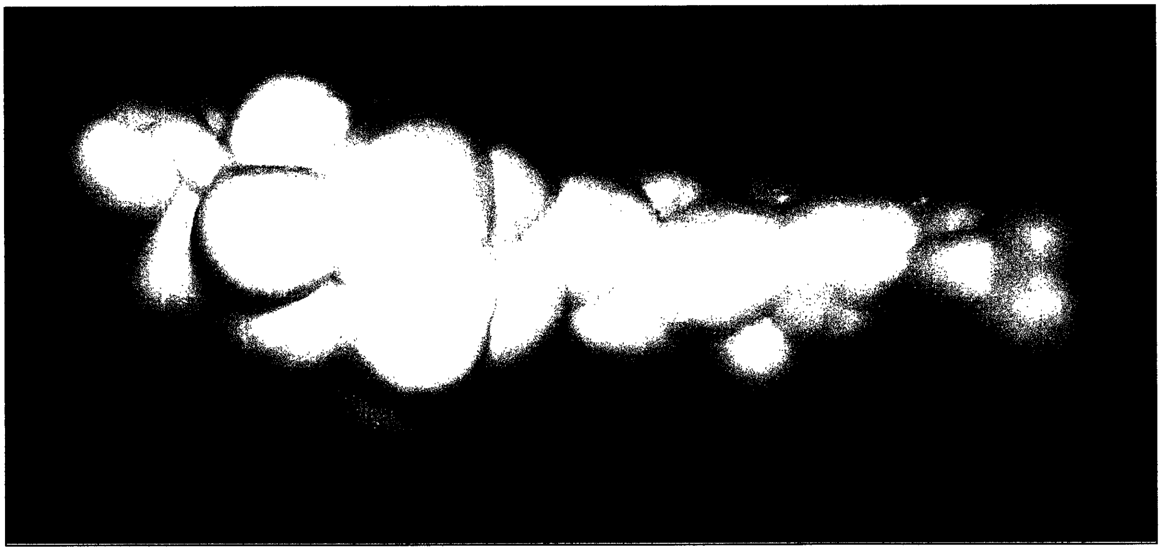

Figure 9: Experimental Master Part. The part shown in this figure is the master part made for the experiment with the multi-part poured molding and casting technique. It has features, such as the rounded ball-like geometry and twisted nature of the overall part, that are representative of the master part for a collagen protein chain.

The master part shown in Figure 9 was made from a thermoplastic called Friendly Plastic, available at retail arts-and-crafts stores. The plastic comes in small, white, hard, beaded form and softens when heated in boiling water. The beads are introduced into a container of boiling water and allowed to sit until they change from their initial white color to a translucent color. By the time the color change occurs, the beads have stuck together in a large mass and can be removed from the hot water with a utensil. The mass of softened plastic can then be handled and shaped into any shape the user desires. As the plastic cools, it hardens back into the white plastic it was in beaded form, only in the shape the user has formed out of it while it was soft. The hardened shape can be resoftened and reshaped as necessary by reintroducing it into boiled water.

The master part shown in Figure 9 was made by rolling up balls of the Friendly Plastic and sticking them together until the part was completed. The master part was difficult to make at times because of the tendency of the plastic to flatten on a rounded surface if it was placed on the table before it hardened sufficiently. At the same time, we didn't want the plastic to harden because the individual balls would only adhere to one another if they were in the softened state. Working with a partner is definitely recommended if trying to make complicated shapes such as the one made for the experimental master part.

Once the experimental part was created, we were ready to pour the mold material around it. In order to pour the mold material around the experimental master part, a support system to elevate the part from the bottom of the mold box was necessary. A few small holes were drilled into the experimental master part so that two appropriately bent paper clips could be inserted to hold the part off the bottom of the mold box. See Figure 10 for a picture of the suspension set-up.

Figure 10: Suspension Set-Up for the Experimental Master Part. Bent paper clips were used to elevate the experimental master part from the bottom of the mold box.

The part was then placed into a small plastic wallpaper tray, ready for the mold material to be poured over it. The mold material was measured out using a digital scale and a mixing bucket. The two components of the PlatSil® 71-40 RTV mold material were mixed thoroughly for approximately 5 minutes, paying special attention to scrape the sides and bottom of the mixing bucket to ensure good mixing. The mixed material was then poured into the tray over the experimental master part. The PlatSil® 71-40 was left to cure in the tray for 48 hours, 24 more hours than the minimum demold time, at room temperature. Figure 11 shows the cured poured mold before the part removal process began.

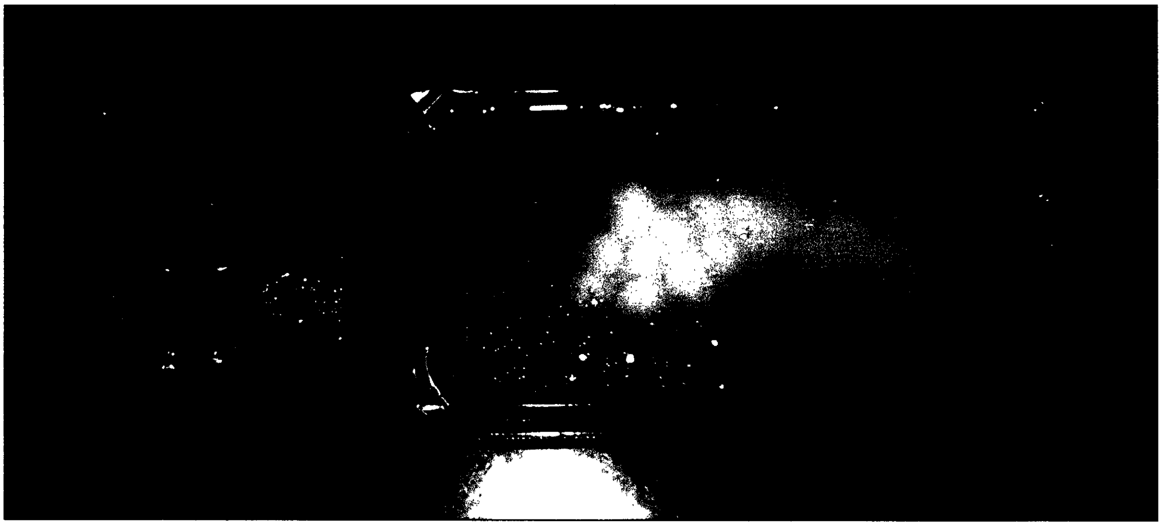

Figure 11: Cured PlatSil® 71-40 Poured Mold with Embedded Experimental Master Part Before Part Removal Process. The white experimental master part is shown embedded within the RTV mold material before the removal process began. This is a top view of the mold.

The mold was then removed from the tray serving as a mold box. Figures 12 through 15 show the mold from different angles after it was removed from the mold box.

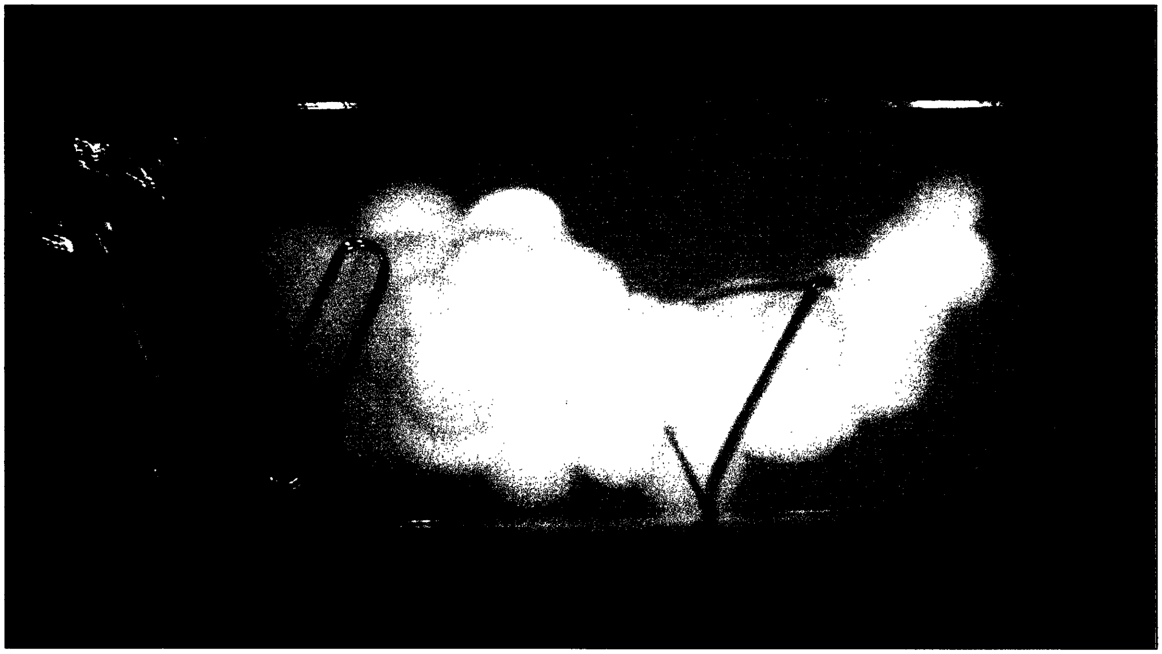

Figure 12: Bottom View of the Solid Poured RTV Mold Before Master Part Removal. This is the bottom view of the mold. The paper clip support system is clearly visible here.

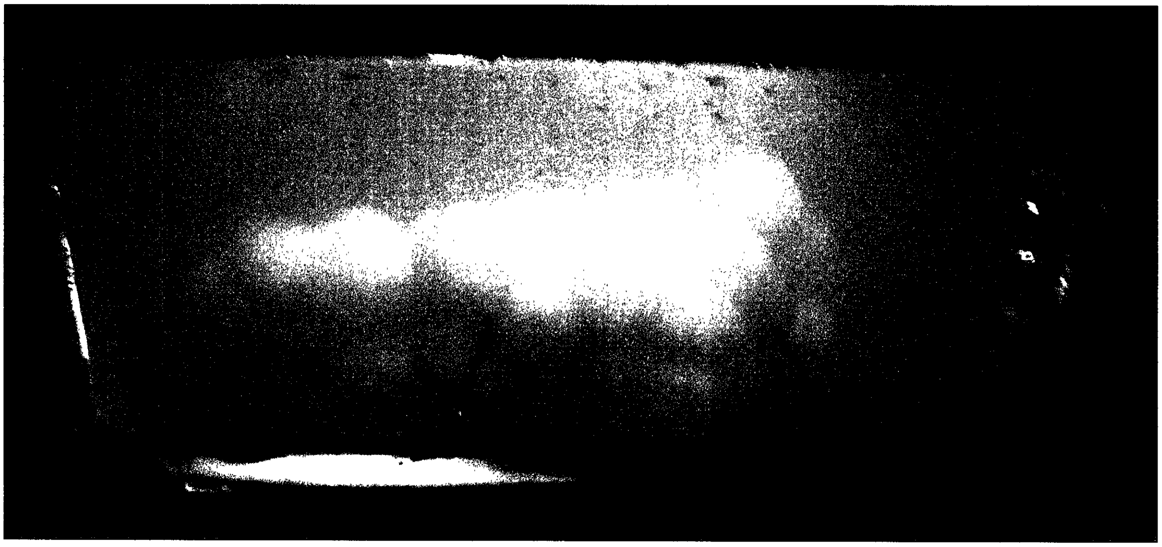

Figure 13: Back View of the Solid Poured RTV Mold Before Master Part Removal. This is the back view of the mold. The paper clip support system is clearly visible here.

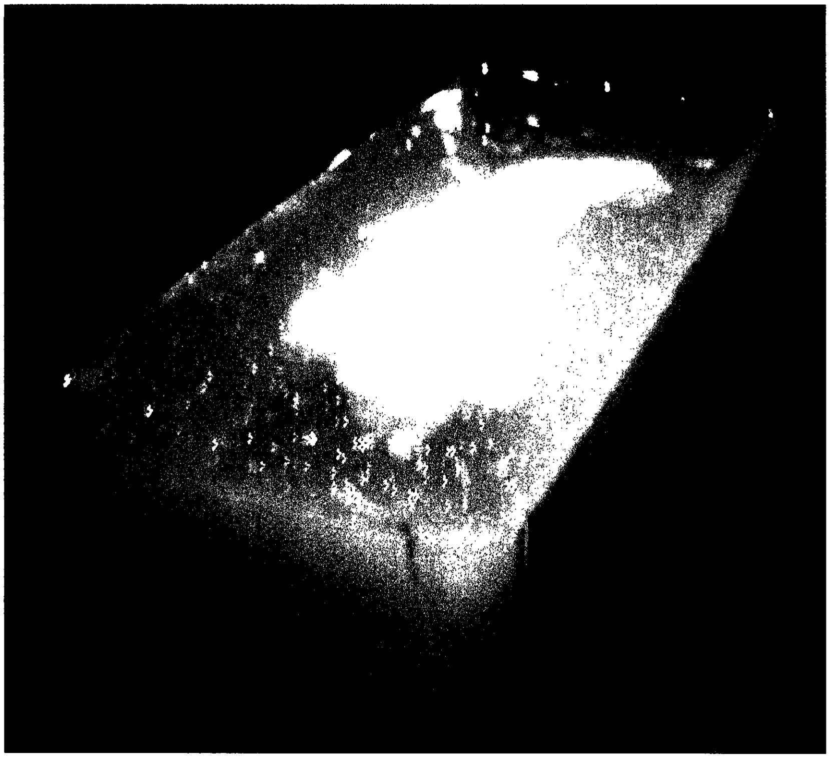

Figure 14: Side Isometric View of the Solid Poured RTV Mold Before Master Part Removal. This is the isometric view of the mold. The depth and overall shape of the mold can be clearly seen in this view.

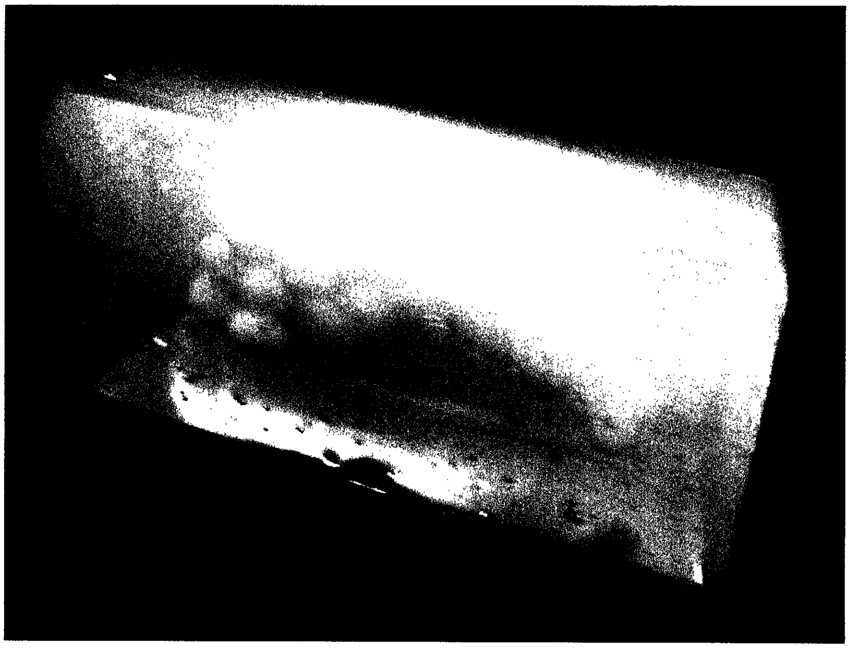

Figure 15: Top Isometric View of the Solid Poured RTV Mold before Master Part Removal. This is the isometric view of the mold. The depth and overall shape of the mold can be clearly seen in this view.

Once the mold had cured, the part removal process could begin. At the suggestion of Professor Gossard, the removal process was designed to leave a mold that would peel away from the part much like the peel is removed from a banana. With this basic parting line design in mind, the cutting lines were scored in the mold with an extremely sharp X-ACTO knife. It became immediately obvious that some excess material on the extreme left of the experimental master part, as shown in Figure 11, would need to be removed before the part removal cuts could be made. The excess material was removed and the scored lines reexamined.

After completing most of the removal cuts, it became obvious that some more bulk material would need to be removed from the top and side of the mold in order to get into the curved area of the experimental master part. To maintain structural integrity of the mold, however, only a portion of the material in the curved section was removed. Figure 16 below shows the final mold with the necessary material removed and the part removal cuts made after the part was removed from the mold.

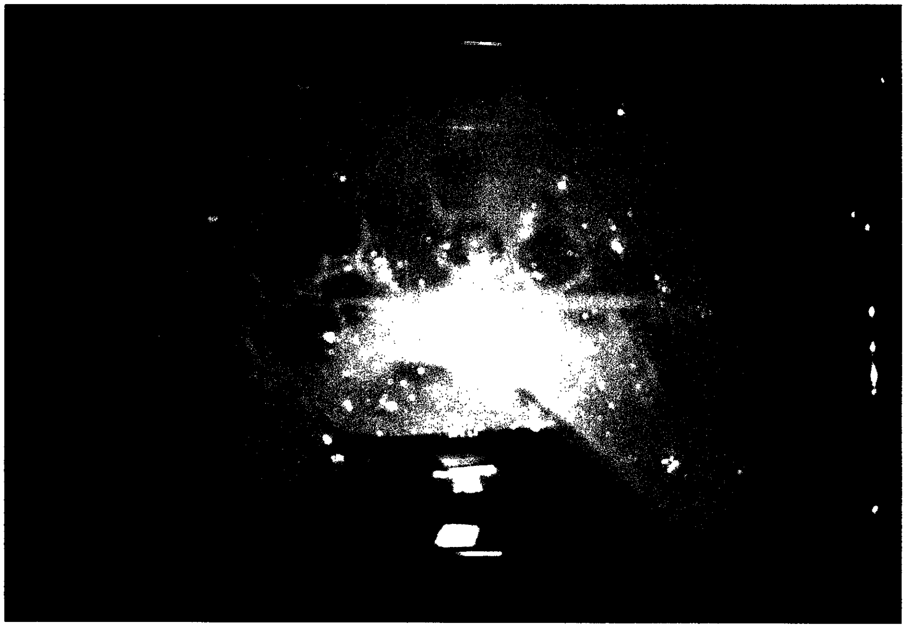

Figure 16: Poured RTV Mold after Master Part Removal. The cavity for the part is visible through the mold. Note the areas of bulk material removed from the right side of the mold and from next to the curved section of the master part cavity.

Several problems presented themselves during the experimental mold production. First, the generation of the parting line was difficult. It was often hard to judge exactly where the master part was within the mold material while cutting, and thus it was often hard to make correct cuts to remove the part. Second, the material was a bit stiff and removing the master part took some force and effort. The effort required to remove the part could lead to accelerated mold wear as parts are made and removed. Third, the material was relatively difficult to cut using an X-ACTO knife. The blade had to be extremely sharp in order to easily cut the material. Frequent blade changes were necessary to ensure easy and clean cutting. It was also difficult to maneuver the blade deep enough into the material to reach the master part. The difficulty in blade maneuvering led to frequent blade slippage. Extreme care had to be taken to ensure cutting was being done safely. Furthermore, the mold was difficult to cast into because we failed to position gates and vents in the mold. Initially the idea was to remove material from the mold to create necessary gates and vents instead of making gates and vents by embedding pieces of material in the mold to be removed during the part removal process. Removing material from the solidified mold to create gates and vents proved to be very difficult. For fear of ruining our mold while trying to remove material for gates and vents, we ended up just using an opening in the mold that had been created by accident during the bulk material removal process. Obviously, this was not ideal and showed that vent and gate locations should be incorporated into the mold from the start.

The multi-part poured mold was not as trivial and easy to create as initially expected, nor was the casting process associated with our experimental multi-part poured mold. The casting process associated with this mold is discussed in Section 5.2.1.

5.1.2 Brush-On Blanket Mold

Due to the difficulties with creating our own multi-part poured mold and the difficulties associated with casting from such a mold, we decided to outsource our mold making task to a professional, skilled molding and casting artisan, Bob Shure at Skylight Studios in

Woburn, Massachusetts. Shure suggested making a mold via a combination of the two-part poured mold and brush-on blanket mold techniques. First he embedded the master part in a bed of clay and created an appropriate parting line via the top surface of the clay. Registration marks were created in the clay surface to allow the mold to be properly aligned during use. After creating the clay bed, parting line, and registration marks, Shure brushed Mold Max 30 mold making material onto the top half of the master part, allowed it to cure, and removed the clay from the other half of the master part. Shure then brushed molding material onto the second half of the master part to create a two-part RTV mold. He created a vent and a gate during this process to allow air to escape as material is poured into the mold .

In order to reinforce the flexible RTV mold, he created a plaster mother mold. The plaster mother mold provided rigid support for the flexible RTV mold and allowed a metal stand to be incorporated with the mold so that it would stand upright during casting. Figures 17, through 21 show the finished mold.

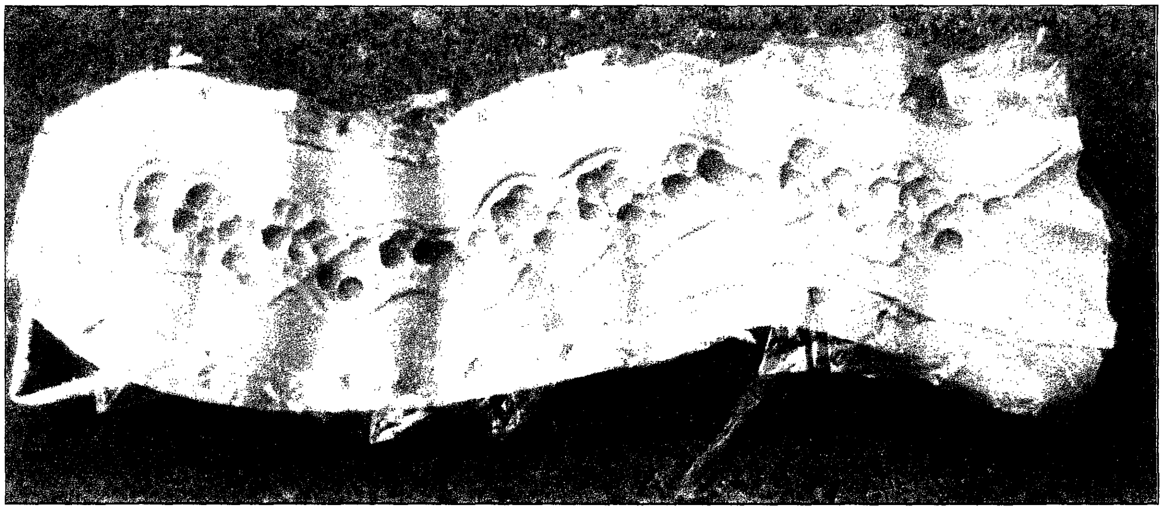

Figure 17: Half of the Final RTV and Mother Shell Mold. The purple material is the RTV Mold Max 30 material. The brown edge is the plaster which had been painted with a shellac for easy mold mating. The divets in the plaster and the ridge around the depressions that create the part cavity act as registration marks. Clear and blue plastic wrap were sandwiched between the plaster and RTV molds to reduce the potential for damage to the plaster mold during casting.

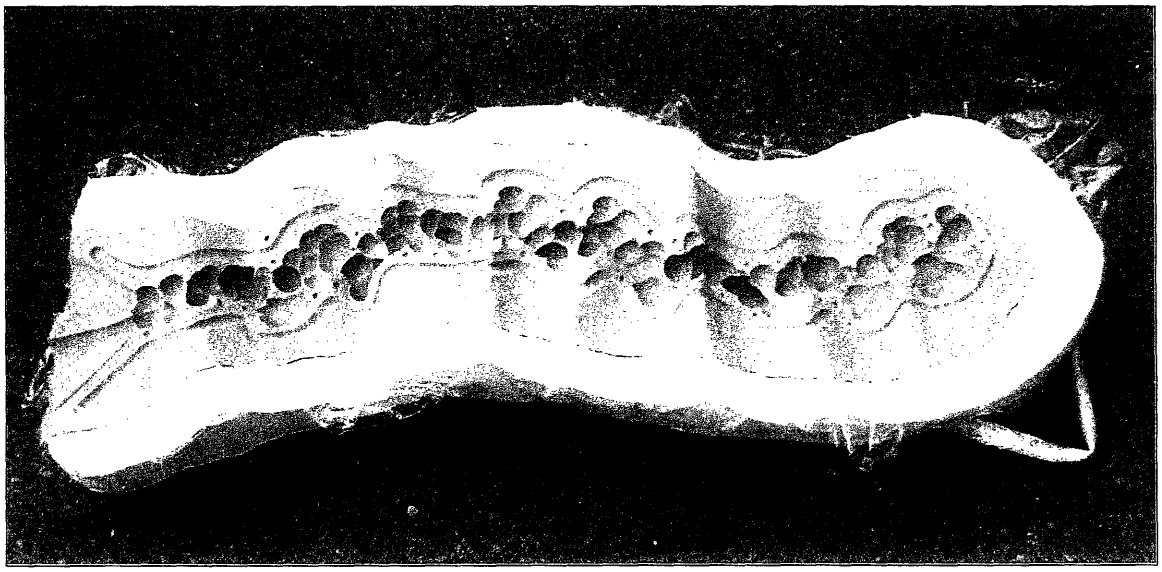

Figure 18: Second Half of the Final RTV and Mother Shell Mold. This is the second half of the final mold that mates with the half shown in Figure 14. Notice the vent and gate location at the left of the mold.

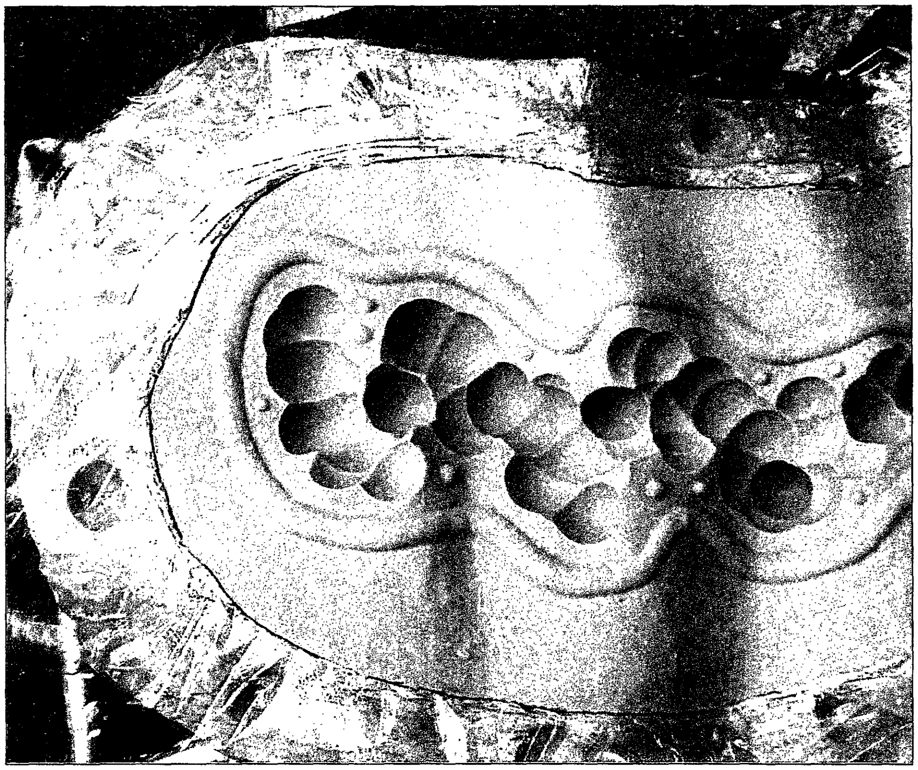

Figure 19: Close-Up View of a Portion of the Mold. The registration marks on the RTV and plaster molds are more clearly shown. Notice the varying depths and overhangs in the part cavity making it clear why it is necessary to have a flexible mold material that allows the cast parts to be released.

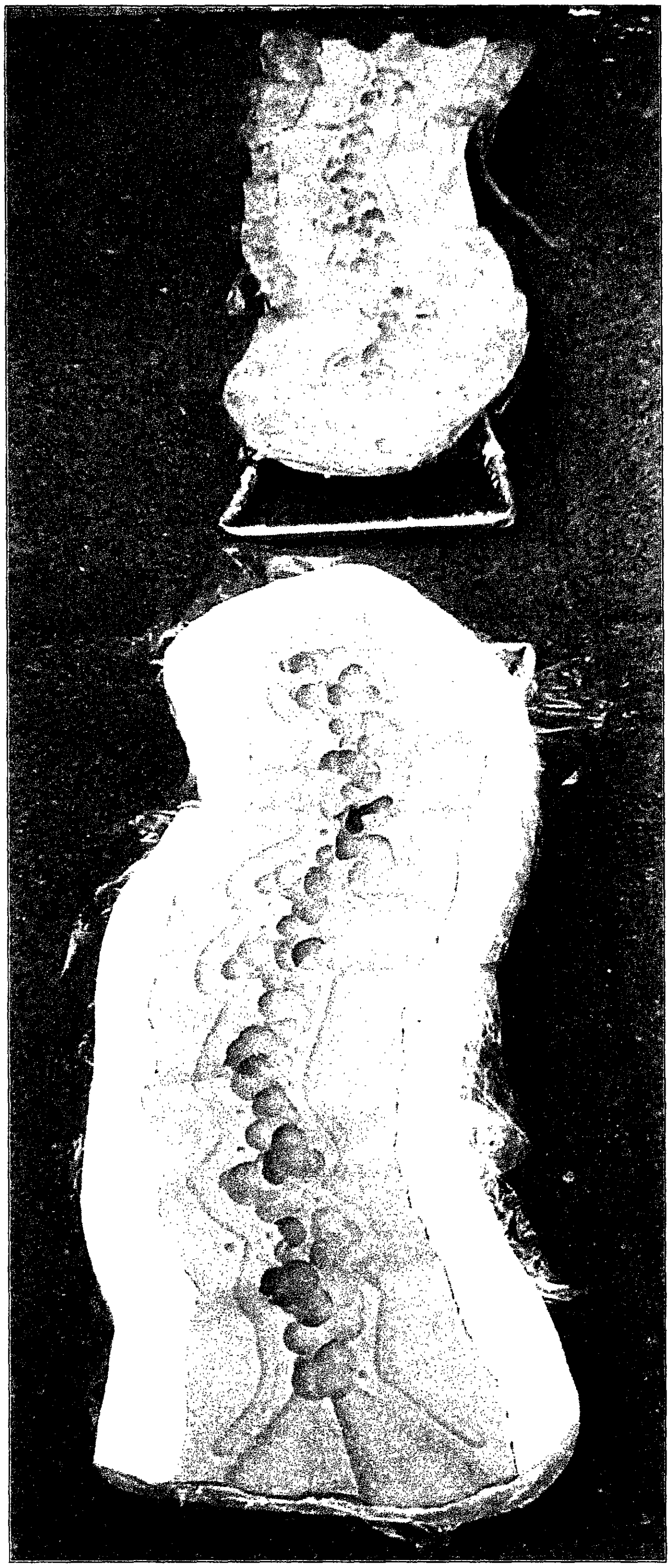

Figure 20: Open Final Mold. Here the two halves of the final mold are opened and laid out in position for the initial casting stage to begin. Section 5.2.2 discusses the method used to cast using this mold.

Figure 21: Closed Final Mold. The mold stands upright. It is in this position for the majority of the casting process. The straps around it provide pressure to keep the mold closed tightly during casting so that material will not leak or flash.

The mold created by Bob Shure at Skylight Studios was far superior to any mold we could have made on our own. Although heavy and a bit unwieldy to work with alone, this mold was much easier to use than the multi-part poured mold we had made in our initial molding experiment. The casting process associated with this mold is discussed in detail in section 5.2.2.

5.2 Casting

Casting is as much of an art as an engineering science and as such there is often a large learning curve associated with casting good parts. The quality of the mold being used is certainly a large factor affecting the relative difficulty of casting, and our attempts at casting certainly reflect this. The evolution of our casting process is discussed in sections 5.2.1 and 5.2.2.

5.2.1 Multi-Part Poured Mold Casting

Casting with the homemade multi-part poured mold proved extremely difficult. Keeping the RTV mold sufficiently closed within the mold box so the casting material would not leak out of the mold was very difficult. It was also difficult to pour the casting material because we failed to place gates and vents in the mold for our initial experiment.

Initially, our casting efforts involved the Castin' Craft Clear Casting Resin, which was the material used with the multi-part poured mold. In order to get a feel for how the material behaved, we cast a rectangular part using a standard, commercially available mold box commonly used for casting paperweights.

First, a layer of Castin' Craft Clear Casting Resin was poured into the rectangular mold and the appropriate amount of catalyst added. The catalyst was stirred into the liquid plastic and allowed to set for approximately 20 minutes while it cured to a jelly-like consistency. At this point, a scrap piece of injection molding plastic we had randomly saved from an injection molding course was placed on top of the layer of jelly-like plastic. More Castin' Craft Clear Casting Resin was poured out into a different container and mixed with catalyst. The plastic/catalyst mixture was then poured over the partially cured, first layer of plastic and then around the piece of injection molding plastic. The second layer was poured until it was level with the top of the black injection molding plastic piece. The material was then allowed to cure for 48 hours until it became completely solid and no longer tacky to the touch.

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After 48 hours, the part was removed from the mold and examined. The part was water clear with the black injection molding scrap piece embedded within the clear plastic. There was no visual evidence of a line between the two layers.

After another 48 hours, the part was placed back into the mold and a third layer of Castin' Craft Clear Plastic Resin poured on top of the existing two layers. The material was allowed to cure for another 48 hours and the part removed for examination. Again, the part was water clear. But there were minimal signs of a visible line marking the division between the second and third layers of plastic. The experimental rectangular part is shown in Figures 22, 23, and 24.

Figure 22: Top View. Top view of the experimental clear part. Notice the water clear material and the embedded black object.

Figure 23: Front View. Notice the absence of a visible line at the bottom of the black embedded object where the first and second poured layers meet. There is, however, evidence of a division between the second and third layer located at the top of the black embedded object. Cloudiness is due to an attempt at sanding, not the material.

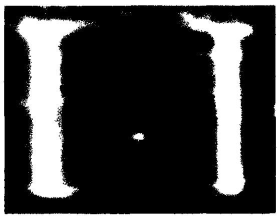

Figure 24: Side View. Side view of the experimental clear part. Notice the division between the third and second layers again.

The experiment with the multiple layer and embedding casting was important when we were intending to embed a ball-and-stick model inside a space-filled model. It was important to understand how to embed something using this plastic material and if there were visual divisions between poured layers in case we needed to pour separate layers to embed the ball-and-stick model. It was also helpful to learn how well new layers of material adhered to previously cured layers. It turns out that the layers do adhere to one another and there is only a slight visual division between layers poured more than 24 hours after one layer has cured.

Having conducted an initial experiment to gain more understanding of the clear casting material, the next step was to use the material to actually cast a part with our multi-part poured mold. First, the Castin' Craft Clear Casting Resin was mixed with the appropriate amount of catalyst in a mixing container then poured into the mold through a hole in the top of the mold that was accidentally created during the bulk material removal process in removing the master part from the mold. The pouring process proved to be tricky and messy due to the poor venting and gating system in the mold. Material leaked out of the mold severely despite being held in the mold box. To inhibit material flow out of the mold, aluminum foil was wrapped around the mold and rubber bands wrapped around the aluminum foil.

The material was left to cure for 72 hours. When the part was demolded, it was incomplete and difficult to remove from the mold. The excessive leakage and the poor mold quality both contributed to demolding difficulties. Figure 25 shows the part created in this initial casting trial with the multi-part poured mold.

Figure 25: Cast Part of the Experimental Master Part. The part shown here is the first part cast from using the multi-part poured mold and the Castin' Craft Clear Casting Resin. Notice that the part is incomplete and has a poor surface finish.

The part created from the multi-part poured mold was not as clear as we had hoped because it had a poor surface finish. The experimental master part's surface finish was a bit rough and every crease in the experimental master part's surface was very apparent in that casting created from its mold. Given the relatively rough surface finish of the collagen protein chain master part, it was apparent that some post-processing of the parts or refining the surface finish of the master part before making the mold would be necessary to obtain water-clear cast parts.

Another problem that was associated with casting with Castin' Craft Clear Casting Resin was the noxious smell of the plastic. The part shown in Figure 25 was cast in the common area of a dorm late at night while people were asleep, multiple windows were opened and a cross breeze induced, but the awful smell of the plastic lingered for at least 24 hours.

The multiple problems encountered while making the experimental multi-part poured mold and the variety of problems associated with casting into such a mold made it clear that we ought to consider a better process for molding and casting our complicated parts. Using the brush-on blanket molding technique and having a skilled artist create a much higher quality mold facilitated the casting process immensely and is discussed in section 5.2.2.

5.2.2 Brush-On Blanket Mold Casting

Casting parts using the mold created by the skilled artist Bob Shure was much easier than casting using the homemade multi-part poured mold, but there were still several obstacles and a learning curve associated with the casting process. Initial attempts were messy and poorly done, but by the third try, good quality parts were being made.

In order to cast a collagen protein chain part using the Smooth-On 320 and the professionally made mold, we first laid out the mold separated into its two halves as shown in Figure 20. The mold was laid out this way initially because pouring material into the deepest pockets before closing the mold and pouring the majority of material allows air to escape more easily and thus minimized air traps in the final part. Plastic wrap (e.g. Saran Wrap) was sandwiched between the plaster and RTV molds on both halves of the mold to protect the plaster part of the mold from potential damage if the casting material were to leak out of the RTV mold.

After laying out the mold, we then mixed the Smooth-On 320 plastic in an 8 oz. batch. Both A and B components were shaken up in their containers as the directions require. Then 4 oz. of part A was poured into a graduated mixing container and 4 oz. of part B was added to the mixing container. The mixture was stirred vigorously for approximately 30 seconds. The transparent brownish liquid mixture was then poured into the deepest areas of the part cavity in both halves of the mold. The material was allowed to cure for approximately 5 minutes at approximately 60°F (we were casting outside on a mild day). When the material began to turn off-white indicating that it was curing, the mold was tipped upright and the two halves were mated. Two compression straps were wrapped around the mold as shown in Figure 21. The compression straps help to keep the mold halves together and minimize leakage and flash.

Once the mold was upright and strapped together, an additional 16 oz. batch of the liquid plastic was mixed and poured into the mold through the gate at the top of the mold. Approximately 3 16 oz. batches were necessary to fill the mold. The mold is filled when the plastic comes up the gate and vent. The material was allowed to cure for 20 minutes and then demolded. The straps were removed, and the mold opened. The part was removed from the RTV mold and the plastic wrap between the plaster and RTV molds was removed.

Some post-processing was necessary on all the parts that were cast for this project. Flashing at the parting line had to be removed and the parting line cleaned up before any painting could be done. Most of the flashing could be broken off by hand, but some of the flashing needed to be broken off using pliers. All the parts were sanded using a Dremel tool to minimize the appearance of the parting line.

The initial attempt at casting using this method was tricky. The compression straps were not tight enough and the mold was not aligned quite right which led to massive leakage and flash. To stop the leakage, additional plastic wrap was wrapped around the mold. Wrapping the mold like this stopped the leakage, but ultimately proved to be a bad idea. The liquid plastic found its way between the plastic wrap and the plaster mold and resulted in mold damage. Much careful removal of hardened plastic on the mold was necessary before additional parts could be cast. Figure 26 shows a finished cast part.

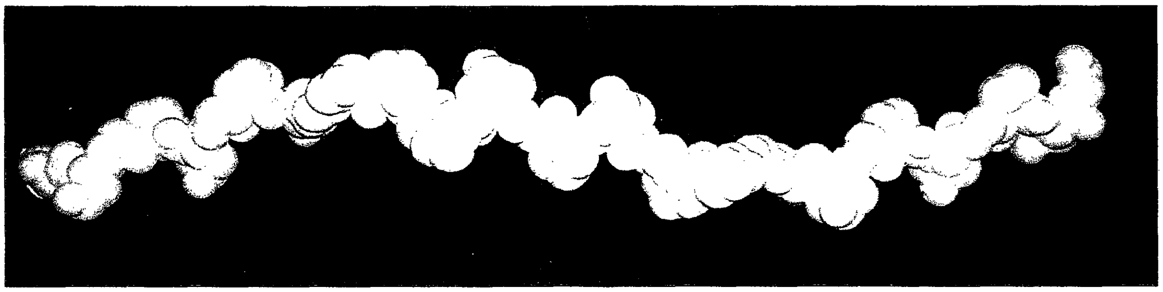

Figure 26: Finished Cast Part. The final casting of one 1CGD collagen-like protein A chain.

Later attempts at casting also had leakage, but to a lesser extent because the compression straps were applied more tightly. Leakage mitigation was accomplished in these attempts by smoothing out the plastic wrap and pressing the two layers together and keeping them away from the mold as much as possible. No further damage was done to the mold in later attempts because the liquid plastic was allowed to flow out of the mold and onto the plastic wrap between the mold layers. The later attempts were also easier because two people were working together to cast the part. Having two people work together made the alignment of the mold halves much easier.

Once the parts were made, they were all cleaned up and painted. Standard latex paint was used to paint the chains. Two chains were painted solid colors and the third chain was painted by residue. Figure 27 shows the final finished painted parts.

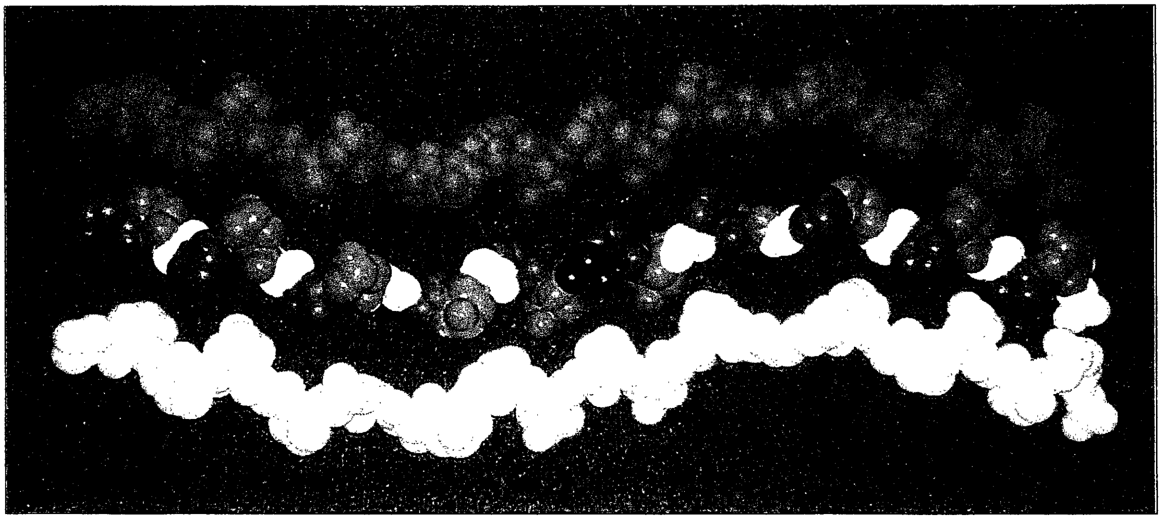

Figure 27: Final Painted Parts. Two of the three chains were painted solid colors to demonstrate the three chained nature of the collagen. The third chain was painted by residue to illustrate the structure of each chain.

The three finished chains can be wound around one another to create the full collagen protein model, as shown in Figure 28.

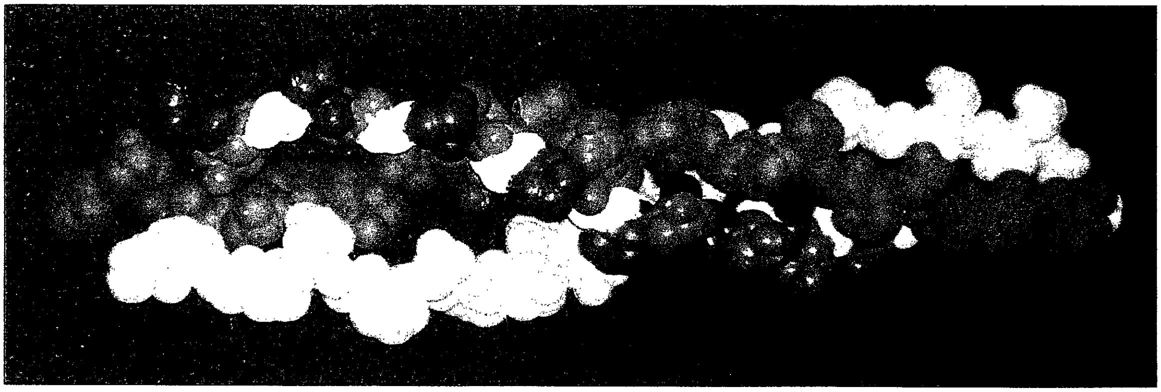

Figure 28: Final Model with Chains Wrapped Together. This figure shows how the three chains wrap together to make the full model.

Assembling the three chains in the correct orientation is not entirely trivial. In order to make assembling the three chains easier, registration marks were incorporated by attaching some Velcro strips in key locations.

After much experimentation and diligence, the final product was completed and is ready to be used in a classroom as a teaching aid.

Chapter 6

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, a meter-scale model of a collagen protein was designed and manufactured for educational use. The A chain of the collagen protein was modeled in SolidWorks, a computer aided drawing program, and an STL file created. The STL file allowed the part to be mathematically sliced into layers so that a three-dimensional printer could manufacture a master part. The master part was then used by artist Bob Shure of Skylight Studios in Woburn, Massachusetts to create a brush-on blanket RTV mold in a plaster shell. The mold was used to cast three chains which were then finished and made to easily wrap together to form the complete collagen protein molecule model.

Much consideration was given to different rapid prototyping processes for the master part production. Research and experimentation was done to determine the best mold making process and technique and become familiar with how to skillfully cast quality parts. Materials requirements were determined and a variety of different materials were considered for the mold and casting processes. Ultimately, Smooth-On Plastic's Mold Max 3 was used for the RTV mold and plaster used as a rigid shell for the RTV mold. Smooth-On Plastic's Smooth-On 320 liquid plastic was chosen as the casting material.

Despite the change in scope and intention of this project, we were successful in manufacturing a functional educational tool. Future investigators may wish to investigate a method by which to manufacture an integrated space-filled and ball-and-stick model. The mold used in this process may be usable for such a project if a method of suspending the ball-and-stick model is devised. The Castin' Craft Clear Casting Resin could be a suitable material to use for such a model. Much attention would need to be given to the design and manufacture of the ball-and-stick model, however. Processes such as injection molding or three-dimensional printing may be useful technologies to consider and explore.

Much has been learned while working on this project and it is our hope that the work presented in this thesis will be of use to future investigators working on similar molding and casting projects.

Chapter 7

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