Embedding of Biliary Calculi in Plastic Materials

A viable solution for increasing their mechanical resistance during sampling

LENUTA-MARIA SUTA[1], PETRU MATUSZ[2]*, ADRIANA LEDETI[1], CODRUT IVAN[2], MARIUS MURARIU[2], MIRCEA CONSTANTIN SORA[3], IONUT LEDETI[1]

[1]University of Medicine and Pharmacy Victor Babes, Faculty of Pharmacy, Eftimie Murgu Square 2, Timisoara, 300041, Romania
[2]University of Medicine and Pharmacy Victor Babes, Faculty of Medicine, Eftimie Murgu Square 2, Timisoara, 300041, Romania
[3]Morfology Centre, Medical Faculty, Sigmund Freud Privatuniversität, Campus Prater Freudplatz 1, A-1020 Wien A-1090 Wien, Austria

This paper deals with an innovative technique regarding the embedding of biliary stones in plastic materials as an alternative and viable solution for increasing their mechanical resistance during processing for preparing the samples for instrumental analysis. The human gallstones provided from a single patient were embedded in two different polymeric plastics. The samples were investigated for their mechanical resistance under surface grinding using a rotating abrasive wheel on a bench grinder, as well lathing on a lathe machine. A discussion for the best method of embedding and the sample preparation is presented.

Keywords: embedding, biliary stones, plastic polymers, mechanical stability

Biliary calculi, commonly known as gallstones, are crystalline solid formations that appear primarily in the gallbladder and are formed from bile components. Gallstone disease is an extremely common disorder that continues to affect humans from the Egyptian days. Gallstones were found during autopsies on king's mummies. Nowadays, 10 to 15% of the adult population is dealing with this disease [1].

The bile duct system (intrahepatic and extrahepatic) [2,3] carry bile from the liver parenchyma in the second part of the duodenum, having at extrahepatic level the gallbladder where bile accumulates and concentrates between periods of active digestion. So, the main purpose of gallbladder is to store and slowly release bile into the digestive system in order for the body to process fats. If at the end of the digestion process, the gallbladder is not emptied out, the remaining bile can become over-concentrated in cholesterol and this tends to lead to the development of gallstones [4].

Identifying the components of gallstones has become essential over the last years because the underlying cause of formation can be fundamental in the treatment course [4]. Four main etiological factors attributed to this pathology: the first, cholesterol hypersecretion into the liver that can lead to supersaturation of the bile and in its turn this can cause gallstone formation. The second is a gall bladder malfunction that leads to a mobility issue and a poor function of the mucosa causing an impaired bile concentration and a disturbance in the enterohepatic circulation. The third factor is an increased crystallization and nucleation of the cholesterol in the bile and the fourth are the disorders associated with a slow intestinal transit that can lead to diminished lipid absorption [5].

Usually, gallstones are classified as cholesterol stones, black stones and brown pigment stones, the last two being included in the pigment stone category. The main difference between cholesterol and pigment stones is based on the cholesterol proportion: more than 70 % in the cholesterol stones compared to 25-30 % in the pigment stones [6].

Black pigment stones consist of bilirubin in a polymerized form alongside varying amounts of calcium salts. These are mainly found in patients that suffer from hepatic cirrhosis or hemolysis. In comparison, brown pigment stones consist mainly of amorphous material, bile pigment, calcium salts and mucus glycoprotein and are often associated with a biliary infection [7]. Although calcium bilirubinate is the major component in both categories, calcium carbonate and calcium phosphate are contained in black pigment stones and rarely found in the brown ones in which calcium fatty acids can be encountered [6].

However, in developed countries around the world, the most common type of gall bladder stone is made out manly of cholesterol (over 70 %). These are further divided in pure cholesterol stones, mixed stones and combination stones. Pure cholesterol stones contain over 95 % cholesterol, with a small pigmented center, radial internal structure with a round or oval shape and a color that varies from white to yellow. Mixed or combination stones have two layers: a pigmented one and a cholesterol one, the position of which determines the brownish to dark brown color, their shape being similar with the pure cholesterol stones [6].

Regarding the risk factors regarding the gallstone formation process, there are a few worth mentioning, such as: genetics, family history of gallstone disease being an indication of increased possibility of biliary calculi formation, or race, the risk diminishing from the Native American Indians, with the highest prevalence, to Africans that have the lowest risk levels. The age is also an important factor, alongside the person's sex, women being at a disadvantage, mainly because of the high levels of hormones during pregnancies. Obesity, rapid weight loss, diet and physical activity are a few more important aspects that should not be overlooked [8].

Despite the research and advances being made in the prevention of biliary calculi formation, these continue to play an important part in various cases of morbidity and mortality around the world. The presence of gallstones may result in acute cholecystitis, gallstone ileus, ascending cholangitis or even pancreatitis [9]. In order to avoid these serious complications, physicians have to determine the treatment course (therapeutically or surgically), thus identifying the exact composition and formation processes of gallstones are essential for a successful outcome [4].

* *e-mail: matusz@umft.ro*

Sample	Type of embedding	Colour	Embedding	Machine tool sampling	Processing direction
1A	Glycol Methacrylate Technovit® 4173	Blue	Partly	-	-
2A				RAW	*
3A				LATH	*
4A				RAW	#
5A1		Yellow	Partly	LATH	*
5A2			Total	LATH	#
6A1			Partly	RAW	*
6A2			Total	RAW	#
1B	Biodur epoxy	Red	Partly	LATH/RAW	#/*
2B				RAW	#
3B				RAW	*
4B				RAW	*

Table 1
ANALYSED SAMPLES AND MECHANICAL PROCESSING OF THE EMBEDDED GALLSTONES

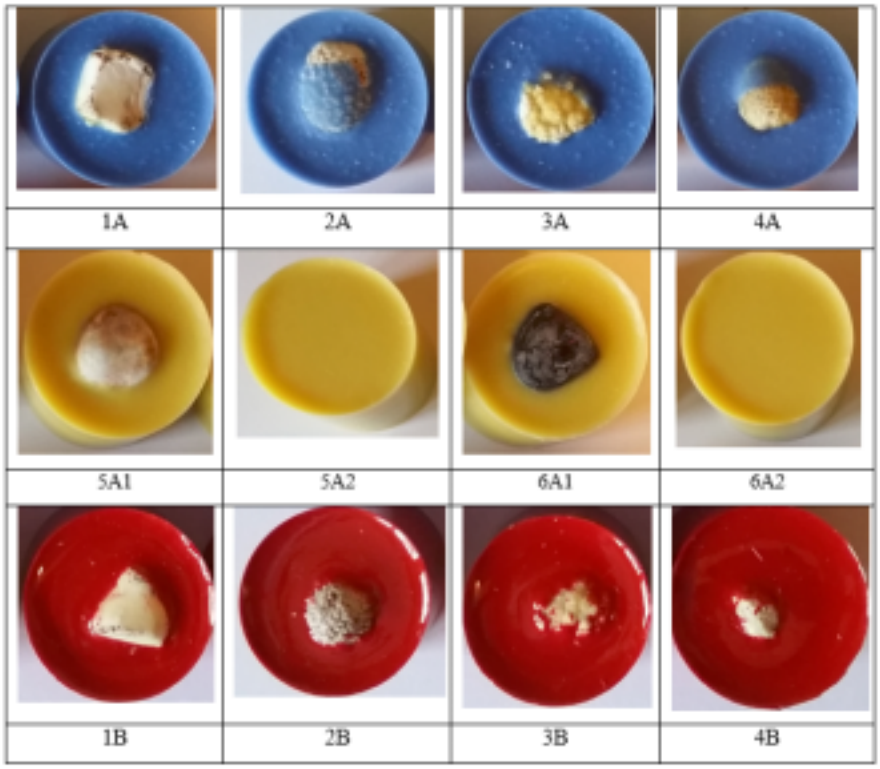

Fig. 1. The aspect of the embedded gallstones. [Color figure can be viewed in the online issue, which is available at www.revmaterialeplastice.ro]

The long-term preservation of tissue and anatomical teaching is realized in our days by using the plastination of body parts using multiple techniques [10-17]. The plastination techniques present some advantages, but some disadvantages too [3,15,18]. Another name for plastination is "forced polymer impregnation" and lead to getting transparency and accurate information for preservation of organs, for well dissected specimens or for body slices [15,19].

As previous stated, it is of highly importance to determine the composition of gallstones, in correlation with the lithogenesis. In order to evaluate the composition of a stone, sampling is done by a concrete separation of the central part of the stone, median and outer one. Since the hardness of the gallstones is decreased, we proposed a viable solution for increasing their mechanical resistance during sampling, namely the embedding in two types of polymers. A discussion regarding the improvement of mechanic stability *vs.* sample preparation is carried out.

Experimental part

Materials and methods

Twelve similar gallbladder stones were investigated. Gallbladder stones (GS) were collected intraoperative from one patient hospitalized at the Department of Surgery II, First Surgical Clinic (Timi°oara, Timis, Romania). All the analyses were carried out with patient's agreement.

The samples were embedded in two types of plastic materials: red epoxy Biodur (Rathausstr.18, 69126 Heidelberg, Germany) mixture: E12 (resin)/ E6 (hardener)/ E600 (accelerator), with a hardening time of 24h and yellow/blue Glycol Methacrylate Technovit® 4173 (Kulzer GmbH, Wehrheim, Germany), with a hardening time of 15 min.

The gallstones were embedded in cylindrical polymeric shapes. The final embeddings were of different height, with a diameter of 35 mm.

The samples were prepared as follows: 4 stones were embedded in red epoxy Biodur, 4 were embedded in yellow Glycol Methacrylate Technovit® 4173 and 4 in blue Glycol Methacrylate Technovit® 4173 (table 1).

The samples were mechanically scraped by friction at 800 rot/min using a rotating abrasive wheel on a bench grinder (RAW) or cut on a lathe (LATH). The processing direction of the samples are presented in Table 1 as follows: with "*" if the process started from the visible stone part, and with "#" if the process started from the non-visible stone part.

Results and discussions

After embedding, the samples were allowed to harden. In order to determine the effect of polymer type over the stability of the gallstone, two different type of polymers

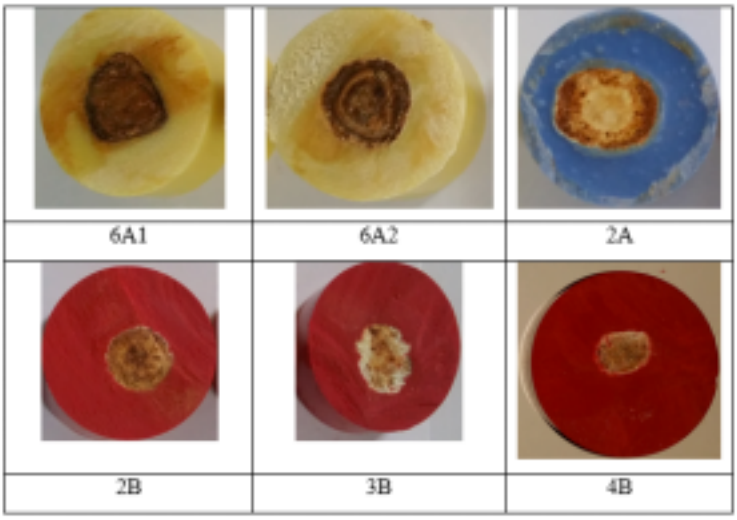

Fig. 2. The aspect of the mechanically processed samples. [Color figure can be viewed in the online issue, which is available at www.revmaterialeplastice.ro]

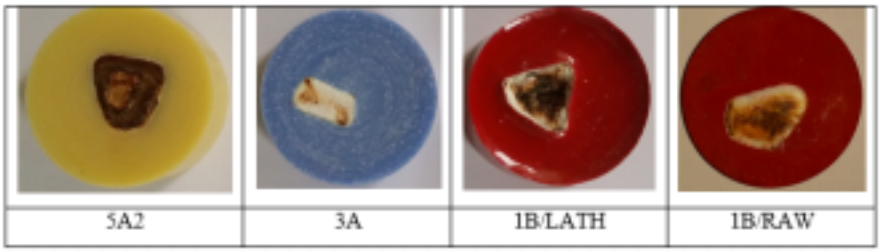

Fig.3. Lathe cutting of the samples 5A2 and 3A and the aspect of the sample 1B under lathe fixation and later RAW processing [Color figure can be viewed in the online issue, which is available at www.revmaterialeplastice.ro]

were used: epoxy resin with a longer hardening time (24h) and Glycol Methacrylate with a short hardening time (15 minutes). The embedding behavior was different for the two polymer types. In the case of the Glycol Methacrylate Technovit® 4173 polymer (samples 1A-6A), due to the rapid polymerization, the embedding of the stones was realized as desired: six gallstones samples were partly embedded (1A-5A1; 6A1), and two were totally embedded (5A2 and 6A2). For these stones, no migration or position change of the gallstones was noticed during sample preparation. In contrast, when using Biodur epoxy resin, a complete embedding of the gallstones couldn't be achieved - after 24 h, the separation of the polymer matrix *vs.* gallstone occurred, due to the difference of densities of the samples. Following this, only partial embedded samples were obtained (fig.1.).

The embedding techniques were required, since the mechanical stability of the gallstones is reduced, the samples being friable during sampling. In order to obtain solid samples from the outer, median and inner part of the stones, their mechanical stability should be increased, and this technique allowed a better processing of the samples.

As mechanical processing of the embedded samples, two highly-accessible techniques were used: polishing on a rotating abrasive wheel on a bench grinder and cut on a lathe. The first technique had the main advantage the rapidness of the process. Samples were polished starting from the non-embedded part of the gallstones (2A, 3A, 5A1, 6A1, 3B, 4B), respectively from the solid polymer part (4A, 5A2, 6A2, 1B, 2B), in order to determine if the stability of the stone is influenced by the complete embedding or not. All the samples revealed a good mechanical stability, in all cases the starting direction of polishing didn't influenced the mechanical stability of the samples. The samples based on Glycol Methacrylate polymer showed a greater resistance towards polishing, the final aspect of the sample being rough, while the ones based on Biodur resin being soft and easy to cut, but the gallstone is somehow impregnated with resin traces (fig.2.).

The mechanical processing using a lathe determined a better cutting of the polymeric material (fig.3.), in the case of Glycol Methacrylate embedding (samples 5A2 and 3A). The Biodur resin embedded samples couldn't be processed by this technique due to the fact that the softness of the polymeric matrix determined the crushing of the stone, during the in place fixation of the workpiece in the machine tool (Sample 1B).

These results showed that the lathe cutting is more accurate in the case of Glycol Methacrylate embedding, while being inefficient in the case of Biodur resin matrix. After the lath cutting trial, the 1B sample was efficiently processed by rotating abrasive wheel on a bench grinder.

Conclusions

In all cases, good results were obtained. Both embedding methods showed some advantages and disadvantages. In the case of Glycol Methacrylate embedding, the main advantage is the rapid polymerization and the stability of the gallstone position in the matrix, while in the case of Biodur resin, the advantage is represented by the rapid sample cutting on the rotating abrasive wheel.

The colour of the embedding matrix polymer does not influence the hardening behavior, but it should be done in agreement with the aspect of the gallstones: for yellow or light colours, blue or red matrixes should be used, while for brown stones, yellow matrix is more suitable.

The best results were obtained when preparing partly-embedded stones in Glycol Methacrylate polymer and the processing is carried out using a lathe, even if requires more time.

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