

Investigation of Biological Activity, Open Porosity and Water Absorption of Ternary Polymeric Blend (Polymethylmethacrylate / Starch) used for Bone Scaffold

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Summary

In this study a fabrication of three groups of biological system for bone scaffold, which consist of first group samples: Polymethylmethacrylate (PMMA type (Re-Acromed) / % X acrylic resin (Type: ORT-365), second group samples (acrylic resin (Type: ORT-365) / % X PMMA type (Re-Acromed)), with selected weight percentages of second material are (5, 10, 15 and 20 % wt.) and third group (PMMA type (Re-Acromed) / 15 % wt. acrylic resin (Type: ORT-365) / % X starch), with selected weight percentage ratios of starch are (2, 4 and 6 %wt.). *In vitro* biological activity and water absorption have been studied. The results clarified that the *in vitro* biological activity test showed that all fabricated samples have no biological influence on the gram positive bacteria *Bacillus subtilis* and gram negative bacteria *Escherichia coli*, the results show that the first group of polymer blend have low open porosity and water uptake capacity than a second group. The increasing in starch weight fraction for the third group led to increasing in open porosity and water absorption.

Keywords: Polymethylmethacrylate, Starch, Water absorption, Biological activity

Introduction

Bones play an important role in our bodies, providing the ability to perform daily activities. They are also responsible for mineral storage and red blood cell production. When bones fail to repair themselves due to severe injury or disease, a replacement surgery can be performed to replace the bone defect with a bone substitute (i.e., Bone, as a functionally smart tissue, is capable of healing and remodeling in the case of limited bone defects) Tables, (1 and 2) .

Polymethylmethacrylate (PMMA) is non-adhesive acrylic polymer that is widely used in orthopedics as bone cement for implants. The orthopedic use of PMMA cement was introduced in the early 1960s by Chanley and this was the first cement used for spine applications. PMMA has been widely investigated as a bone cement for over 40 years and many injectable PMMA cement formulations designed for vertebral body applications are commercial available. PMMA bone cement occupied an unquiet space for

several decades due to its desirable characteristics, e.g. sufficient strength to provide mechanical support, moldable to fill complex defects Tables, (3 and 4). A polymer blend is a member of a class of materials in which two or more polymers are blended together to create a new material with different physical properties. They combine in an advantageous manner the properties of the alloying components and in some cases the properties of the blends polymeric are superior to those of their individual components, blending of polymer is a technological way for providing materials with full set of desired specific properties at the lowest cost, e.g. a combination of strength and toughness, strength and solvent resistance, etc. Blending also benefits the manufacturer by offering improved process ability, product uniformity, quick formulation changes, plant flexibility and high productivity, i.e. Polymer blending is one of the most common techniques employed for developing new polymeric materials [5]. Starch is one of the most abundant and cheap

polysaccharides produced by various plants as a source of stored energy. It is found in plant roots, stalks, and crop seeds and is produced by staple crops such as rice, corn, wheat, tapioca, and potato. It is deposited in the chloroplast of plant cells as insoluble granules composed of α -amylose (normally 10–30%) and amylopectin (normally 70–90%), depending on its source. Starch granules are hydrophilic and show strong intermolecular associations through hydrogen bonds formed by hydroxyl groups on the granule surface. Starch-based materials have been widely used as biomaterials in bone replacement and fixation and for developing tissue engineering scaffolds. In addition, some studies have shown that starch is beneficial in drug delivery applications [6]. In most studies, PMMA was used as a matrix material alone and was reinforced by some substances such as (fly ash, fly dust and ZrO_2 , and Y_2O_3) or a binary phase composition of PMMA and other polymeric materials such as HDPE and the mechanical and biological characteristics were studied for them Tables, (7, 8 and 9). The aim of this study is to prepare three type of polymeric blends with PMMA as the base material which was used to improve antibacterial activity of biomaterials, firstly via blending different types of PMMA material, secondly via blending another type of chemically different polymers with optimum sample of the first groups as a tool for tailoring characteristics of the products with optimized material properties..

Materials and Methods

Preparation of Blends: Cold-cure acrylic for bone scaffold, as resin material manufactured by (company of Re- Acromed) was supplied from UAE, and another type of acrylic resin manufactured by (ORT-365) from Ankara. Tables (1 and 2) show the technical information of PMMA (Type: Re- Acromed) and acrylic resin (Type: ORT-365) respectively according to the product company

Table, 1: Technical information of PMMA (Type: Re- Acromed) according to the product company.

Moulding Consistency	
Mixing Ratio	5 wt. powders, 3.5 wt. liquid
Mixing Time	Approximately 30 seconds
Working Time	Ends after about 2 minutes
Curing Time	Approximately 14 minutes

Table, 2: Technical information of acrylic resin (Type: ORT-365) according to the product company

Viscosity at 20 °C 00/350 mPa.s	
Resin/ Hardener	100/2
Time for Work at 20 °C	8-10 min.
Time for separation	15 min
Hardness shore D	80-85

Corn starch, the starch that was utilized in the current work was obtained from Daejung chemicals and metals Co., LTD (Korea). Table, (3) illustrated the properties of starch that's used in present research according to the product company

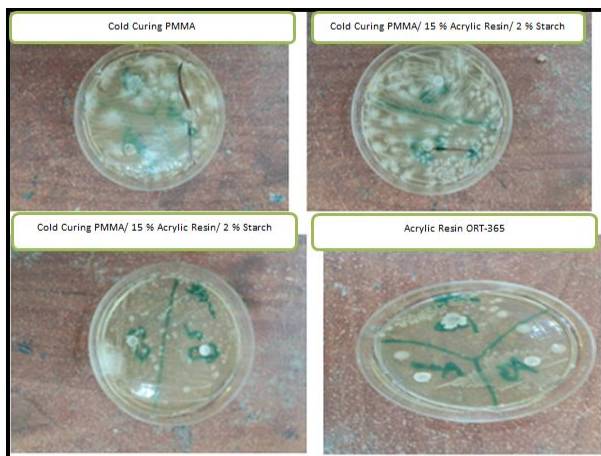
Table, 3: Properties of starch according to the product company.

Formula	$C_6H_{10}O_5$
Ph	4-7
Moisture Content	$\leq 15\%$

PMMA (Type: Re- Acromed) specimens were prepared by hand lay-up molding (5 Wt. powders) with (3.5 Wt. liquid), of methyl methacrylate monomer, as well as according to manufacturer instructions for (Type: ORT-365), the mixing ratio of acrylic resin to hardener is (100/2). Two types of polymeric blends were prepared. The first type was prepared by weighing amount of PMMA (Type: Re- Acromed) as a basic substances reinforced by (5, 10, 15 and 20 %Wt.) of acrylic resin (Type: ORT-365) were uniformly mixed manually until the mixture got to homogenous state. The mixture was poured into the mold properly to get the final uniform specimens. After the molding process, for the purpose of facilitating the exit of samples from it, also to get smooth sample surface and free from defect. This mixture has been left in the mold for 48 hours at room temperature to solidify it. After that, the cast is placed inside an oven at 50 °C for 3 hours. Finally, it was left for 72 hours at room temperature. This process is important to reveal complete polymerization. To prepare the second type of polymer blends, the same pre-preparation steps were repeated except for the proportions of the mixing components mentioned above, they have been replaced by one another. A ternary phases of polymeric materials were prepared by blending (PMMA type (Re-Acromed) / 15 % wt. Acrylic

resin (Type: ORT-365)) with (2, 4 and 6) % wt. of starch .

In vitro Biological Activity Test: The antibacterial activity of PMMA (Type: Re-Acromed), acrylic resin (Type: ORT-365), PMMA(Type: Re – Acromed) / 15% wt. Acrylic resin (Type: ORT -365) and (Re – Acromed PMMA/ 15% wt. ORT -365 Acrylic resin/ 2% wt. starch) specimens, were carried out by disk diffusion method, with a (6 mm) diameter and (2 mm) thickness. The bacterial microorganism which were utilized in this test include gram positive bacteria *Bacillus subtilis* and gram negative bacteria *Escherichia coli* (E. coli). Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultured were swabbed on these plates. All samples were placed in the nutrient agar plate and kept for incubation at 37 °C for 24 hours. Zone of inhibition were measured. The experiments were repeated four times and mean values of zone diameter were presented,(fig.1) illustrates zone of bacterial inhibition by prepared disk.



Figure, 1: Shows zone of bacterial inhibition.

Water Absorption and Open Porosity Test: According to ASTM D-570 [10], the water absorption and open porosity was carried out by utilizing (Precision balance), manufactured by (Radwag in USA), kind (series) (PS 360/C/1). In this test the rectangular samples with dimensions (10 mm) width, (20 mm) length, and (4 mm) thickness, were immersed in deionized water for 30 days (11). Water absorption and open porosity test was performed for all fabricated samples by

weighing the specimens in three different conditions using a four digit weight scale: The first weight in air, the second weight includes immersion of the specimens through a hook balance in distilled water. In the third weighing, the specimens were immersed in distilled water for 30 days, and during this period the specimens were removed from water at each 24 hours and dryness by using soft cloth, then weighed by using electronic balance of accuracy 10⁻⁴. The percentage of water uptake capacity and open porosity percentage were calculated from the following equations (12).

%Water Absorption =

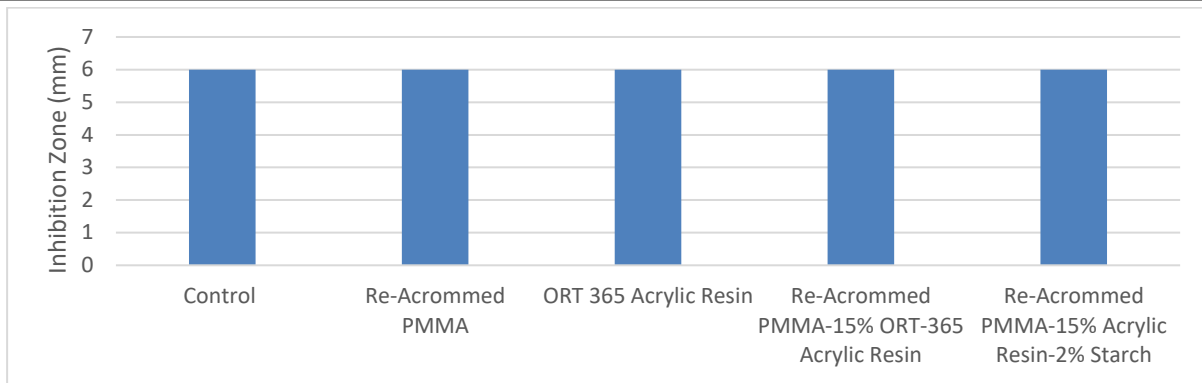
$$\left(\frac{\text{Wet weight} - \text{Dried weight}}{\text{Dried weight}} \right) * 100 \quad [1]$$

%Open Porosity =

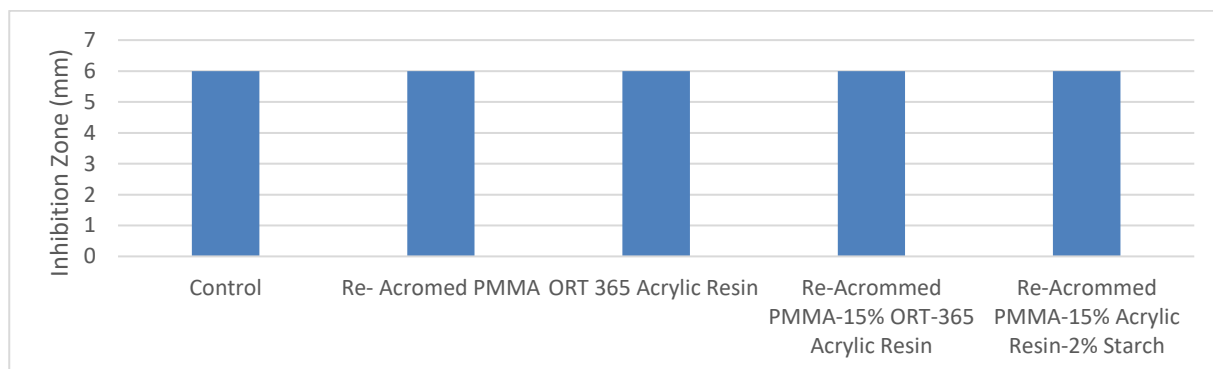
$$\left(\frac{\text{Soaked Weight} - \text{Dry Weight}}{\text{Soaked Weight} - \text{Suspended Weight}} \right) * 100. \quad [2]$$

Results and Discussion

Figures (2 and 3) shows, the biological activity of (Re-Acromed PMMA), (ORT-365 acrylic) and (Re – Acromed PMMA/ 15% wt. ORT -365 Acrylic resin/ 2% wt. starch), used in the current work against gram positive bacteria *Bacillus subtilis* and gram negative bacteria *Escherichia coli* respectively, all samples were kept during incubation periods at 37 °C for 24 hours, the biological activity depend on inhibition zone diameter generated around each disk. So, it can be noted from this figure there is no inhibition zone (bacterial killed zone) around each sample after incubation for 24 hours as compared with control sample. Therefore, these samples can be utilized in bio applications as bone scaffold because these samples do not have biological effect on the bacteria microorganisms (13).



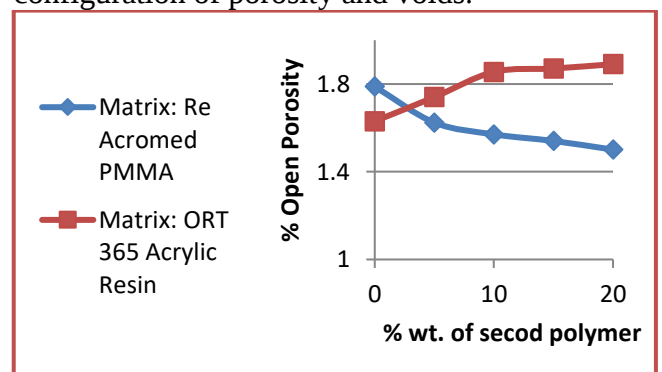
Figure,2: Biological activity of (Re-Acrommed PMMA), (ORT-365 acrylic) and (Re – Acrommed PMMA/ 15% wt. ORT -365 Acrylic resin/ 2% wt. starch) against gram positive bacteria *Bacillus subtilis*. All samples were kept during incubation periods at 37 °C for 24 hours.



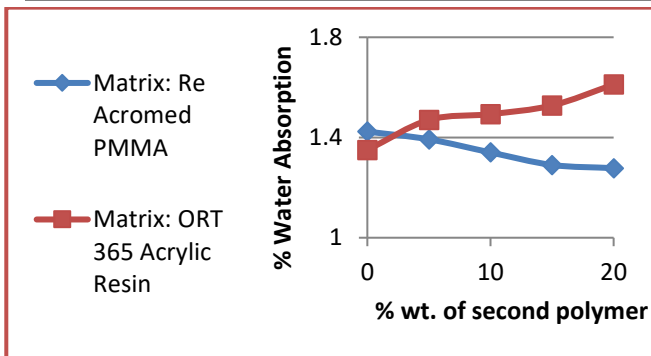
Figure, 3: Biological activity of (Re-Acrommed PMMA), (ORT-365 acrylic) and (Re – Acrommed PMMA/ 15% wt. ORT -365 Acrylic resin/ 2% wt. starch) against gram negative bacteria *Escherichia coli* (*E. coli*). All samples were kept during incubation periods at 37 °C for 24 hours.

Figures (4, 5, 6 and 7) describe the behavior of open porosity and water absorption properties for all fabricated samples of polymeric blend and polymeric composites respectively. From these figures, it is observed, there is a significant difference between first group samples (PMMA type (Re-Acrommed) / % X acrylic resin (Type: ORT-365)), second group samples (acrylic resin (Type: ORT-365))/ % X PMMA type (Re-Acrommed)), and third group (PMMA type (Re-Acrommed) / 15 % wt. acrylic resin (Type: ORT-365)/ % X starch) as composite materials. It has been observed from figures (4 and 5), that the open porosity and water absorption of first group decrease with adding of second phase material in a matrix, as compared with their counterpart of the second group of polymeric blends. The reason behind such a behavior can be attributed to the strong bonding at the interfacial region (good wettability) between two phases of second group of polymeric blends which leads to decrease the micro voids and open spaces.

Therefore, this reason leads to decreasing the water absorption of the first group. However, higher water absorption of second group samples than a first group can be attributed to accumulate the dispersed phase, which increased the free volume and through the configuration of porosity and voids.



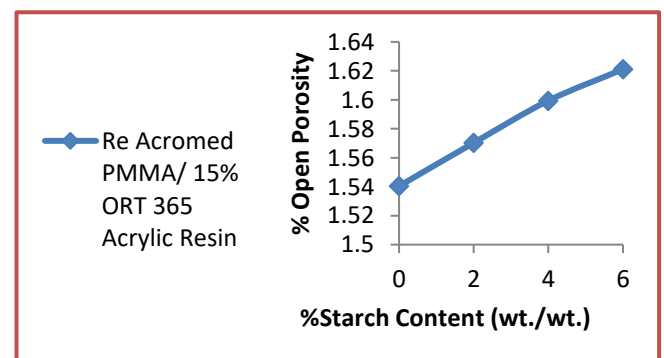
Figure, 4: Open porosity of (PMMA type (Re-Acrommed) / % X acrylic resin (Type: ORT-365)), and (acrylic resin (Type: ORT-365))/ % X PMMA type (Re-Acrommed)) as a function of a second material content in blend.



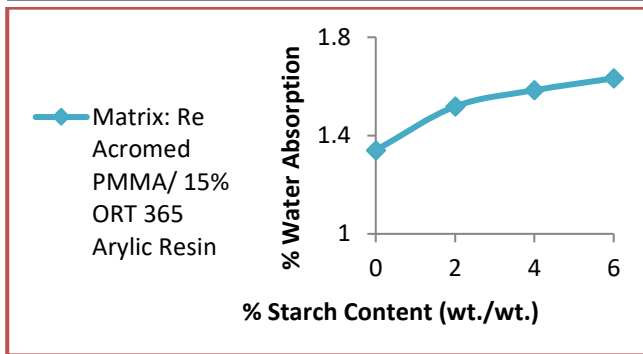
Figure, 5: Water absorption of (PMMA type (Re-Acromed) / % X acrylic resin (Type: ORT-365)), and (acrylic resin (Type: ORT-365))/ % X PMMA type (Re-Acromed)) as a function of a second material content in blend.

The effect of addition of starch on the behavior of open porosity and water absorption properties can be observed from (fig.6 and 7) respectively, from these figures, it can be observed that the open porosity and water absorption increases with an increase in weight fraction of starch material. The increased in water absorption may be due to the hydrophilicity that's high-water sensitivity of starch (6). The high-water uptake capability of the samples generates a rougher surface for cell adheres. In most reports starch material is subjected to reaction with methyl methacrylate to produce starch copolymer (14). As well as, the open porosity increased with an increasing of starch content in the blend, this may be due to an increased in degradation rate of starch. Whereas, starch material is highly hydrophilic in nature due to it's a large number of hydroxyl groups on the backbones. One of major bottlenecks for the application of starch-based products is its great water absorption characteristic. More specifically, the high moisture absorption of starch deteriorates the adhesion with polymeric matrix and then lead to premature ageing by decomposition and loss of strength. It has been reported that the water absorption was strongly dependent on various factors, such as the immersion time, filler concentration, interfacial adhesion and the number of voids formed in the interfacial area. Additionally, it can be also seen that the water absorption and open porosity ratio was also increased as the weight percentage of starch increased (15).

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Figure, 6: Open porosity of (PMMA type (Re-Acromed) / 15 % wt. acrylic resin (Type: ORT-365))/ X% starch) as a function of starch material content in blend.



Figure, 7: Water absorption of (PMMA type (Re-Acromed) / 15 % wt. acrylic resin (Type: ORT-365)/ % X starch) as a function of starch material content in blend.

In the current work, efforts are made to develop PMMA polymeric composites materials which are utilized in the bone scaffold. The following conclusions were drawn based on the results of the tests received in the present work. The *in vitro* biological activity test showed that the all fabricated samples have no biological influence on the gram-positive bacteria *Bacillus subtilis* and gram-negative bacteria *Escherichia coli*. The results show that the first group of polymer blend have low open porosity and water uptake capacity than a second group. Open porosity and water absorption of third group increases with increasing starch content in a matrix. Based on the above, the samples of the third group) (PMMA type (Re-Acromed) / 15 % wt. acrylic resin (Type: ORT-365)/ % X starch) are promising substances in biochemical applications such as bone scaffold.

References

1. Huan Z. , Chu H. K. , Yang J. and Sun D., (2015). "3D Cell Manipulation with Honeycomb-Patterned Scaffold for Regeneration of Bone-Like Tissues", Conference: IEEE International Conference on Information and Automation Lijiang, China, PP: 1680-1686 .
2. Huang B., Caetano G., Vyas C., Blaker J. J. , Diver C. and Bártolo P., (2018). "Polymer-Ceramic Composite Scaffolds: The Effect of Hydroxyapatite and β -Tri-Calcium Phosphate", Materials (Basel) Article, 14, 11 (1):129.
3. M. A. Lopez, Sa Y., Salmon P., Wijn J. R. D. , Wolke J. G. C., and Jansen J. A., (2012). "Bulk Properties and Bioactivity Assessment of Porous Polymethylmethacrylate Cement Laded with Calcium Phosphate Under Simulated Physiological Conditions", Acta Biomaterialia, Elsevier Publisher, 8 (8):3120-3127.
4. Sa Y., Yang F., Wijn J. R. D., Wang Y., Wolke J. G. C., and Jansen J. A., (2016). "Physicochemical Properties and Mineralization Assessment of Porous Polymethylmethacrylate Cement Loaded with Hydroxyapatite in Simulated Body Fluid", Materials Science and Engineering C, Elsevier, 61: 190-198.
5. Hadi A. N. and Olewi J. K., (2015). "Improving tensile strength of polymer blends as prosthetic foot material reinforcement by carbon fiber", J material Sci Eng, 4(2):2169-0022.
6. Park S. B., Lih E., Park K. S., Joung Y. K., and Han D. K., (2017). "Biopolymer-Based Functional Composites for Medical Applications", Progress in Polymer Science, Elsevier Publisher, 68: 77–105.
7. Salih S. E., Olewi J. K., and Talea A. M., (2016), "Effect of Water Absorption on the Compressive Strength for PMMA Nano Composites and PMMA Hybrids Nano Composites Reinforced by Different Nanoparticles Used in Dental Applications", Eng. &Tech. Journal, 34, Part (A) (14):2654-2669.
8. Khalil B. I., Gharkan , Kadhum S. A., Aliwi A. H., (2016). "Study of Some mechanical and physical properties of cold curing Acrylic resin reinforced with particle yttrium oxide", Journal of the college of basic education: Pure science", 23(97):1-12 .
9. Salih S. E., Al.gabban A. M., and Abd Alsalam A. H., (2017). "Preparation And Characterization Advanced Polymer Blend Nano Composite Materials", The Iraqi Journal For Mechanical And Material Engineering, 17(4): 625-638 .
10. Sapuan S. M., Ismail H., and Zainudin E. S., (2018). " Natural Fiber Reinforced

- Vinyl Ester and Vinyl Polymer Composites: Development, Characterization and Applications", 1st Edition, Wood head Publishing Series in Composites Science and Engineering, UK, Elsevier Publisher .
11. Makvandi P., Nikfarjam N., Sanjani N. S., and Qazvini N.T., (2015). "Effect of silver nano particle on the properties of polymethylmethacrylate nano composite network made by in sit photoiniferter-mediated photopolymerization", Bull. Mater. Sci., 38(6):1625-1631 .
 12. Fadhil A. A., (2018). "Investigation of Mechanical Properties for Hybride Polymer Composite Materials", M.Sc Thesis, Department of Mechanical Engineering, University of Al-Nahrain, Iraq .
 13. Talea A. M., (2016). "Development of Polymer and Hybrid Polymer Nano Composites Used as Denture Base Material ", M.Sc. Thesis, Department of Materials Engineering, University of Technology, Iraq.
 14. Zhang O. L., Tian X. H., Sun J. Y., Yuan Y. Z., and Zhang K. T., (2017). "Preparation of starch-g-PMMA, Starch-g-P(MMA/BMA) and Starch-g-P(MMA/MA) Nanoparticles and Their reinforcing Effect on Natural Rubber by latex Blending: a Comparative study", Polymer Science, Series A., 59 (5) : 708–717.
 15. Li M. C. , Ge X. , and Cho U. R., (2013). "Mechanical Performance, Water Absorption Behavior and Biodegradability of Poly (methyl methacrylate)-Modified Starch/SBR Biocomposites", Macromolecular Research, Springer, 21 (7):793-800.

النشاط البيولوجي ، المسامية المفتوحة وامتصاص الماء للخليط البوليميري الثلاثي (بولي مثيل ميثا اكريليت/ النشا) المستخدم في سقالة العظام

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الخلاصة

في هذه الدراسة ، تم تصنيع ثلاث مجموعات من النظام البيولوجي لسقالة العظام ، المجموعة الاولى تكونت من : بولي مثيل ميثا اكريليت (نوع Re-Acromed) ، راتنج أكريليك % X (النوع ORT-365)، اما المجموعة الثانية فتكونت من (راتنج الأكريليك (نوع ORT-365)، بولي مثيل ميثا اكريليت % X نوع (Re- Acromed) (مع نسب مئوية محددة من بولي مثيل ميثا اكريليت (5، 10، 15 و 20% بالوزن) والمجموعة الثالثة (بولي مثيل ميثا اكريليت نوع (Re-Acromed) راتنج الأكريليك 15% بالوزن (نوع ORT-365)، % X النشا) ، مع نسب مئوية من النشا هي (2 ، 4 و 6% بالوزن). تمت دراسة النشاط البيولوجي وامتصاص الماء في المختبر. أوضحت النتائج أن اختبار النشاط الحيوي بالمختبر لجميع العينات المصنعة ليس لها تأثير بيولوجي على البكتيريا إيجابية الجرام (Bacillus subtilis) والبكتيريا سالبة الجرام (E. coli). أظهرت النتائج أن المسامية المفتوحة وقدرة امتصاص الماء للمجموعة الأولى هي أقل مما في المجموعة الثانية. وكذلك أدى تزايد الوزن الجزئي للنشأ في المجموعة الثانية إلى زيادة المسامية المفتوحة وامتصاص الماء.

الكلمات المفتاحية: بولي مثيل ميثا اكريليت ، النشا ، امتصاص الماء ، النشاط البيولوجي.