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Articles and Statements

Novel Hemicyanine and Aza-Hemicyanine Dyes: Synthesis, Spectral Investigation and Antimicrobial Evaluation

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Abstract

Novel hemicyanine dyes and aza-hemicyanine dyes having the nucleus of furo[(3,2-d)pyrazolium; (3,2-d)imidazol] iodide salt were prepared. The electronic visible absorption spectra of all the synthesized hemicyanine dyes and aza-hemicyanine dyes were investigated and in 95 % ethanol solution to evaluate their spectral characterization. The antimicrobial effects of some selected dyes were tested against various bacterial and fungal strains (Escherichia coli, Staphylococcus aureus, Aspergillus flavus and Candida albicans) to assess their antimicrobial (bactericidal and fungicidal) properties. The results discussed in this study revealed that both the spectral characterization and the antimicrobial properties of the examined dyes is highly effected by the type of the X substituted in the phenyl ring system for the hemicyanine dyes and by the type of the phenyl and/or the naphthyl ring system for the aza-hemicyanine dyes. Structural confirmations were identified by elemental analysis, visible spectra, IR and ^{1}H NMR spectroscopic data.

Keywords: cyanine dyes, hemicyanine dyes, synthesis, absorption spectra, antimicrobial activity, aza-hemicyanine dyes.

1. Introduction

Hemicyanine dyes (Kim, 2006; Hammer, 1964; Raue, 1990; Pardal et al., 2002; Vassilev, 2004; Deligeorgiev, 2005; Mazinres, 2007; Nagarajan, Perumal, 2004; Lai et al., 1997; Kabatc et al., 2015; Malegoll et al., 2005; Narayan, Ansari, 2008; Davis et al., 2004) are one of the most widely used and important class of functional dyes. They are used as sensitizers and other additives in the photographic industry, chemosensors, indicator dyes, in optical recording media in laser disks, as flexible dyes, in textile industry, laser dyes, as optical sensitizers and in various other fields, for example dye-sensitized solar cells and dyes with non-linear optical properties. The most important applications for these dyes are in bio-labeling and in medicinal analysis. In addition, hemicyanine dyes (Davis et al., 2004; Shindy, Koraiem, 2008; Shindy et al., 2014; Davis et al., 2006; Wuskell et al., 2006; Banerji et al., 1982; Jha, Banerji, 1985; Jha, 1986; Heilbron, Walter, 1925; Ren et al., 2012; Shindy, 2015; Shindy, 2007) is common fluorescence probe for electrical membrane potential in biochemistry and biophysical area. It is also a very important fluorescence dyes applied in lasers, molecular electronics and nonlinear optical photolimiting devices. Besides,

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hemicyanine dyes (Huang et al, 2002; Antonious, 1997; Kabatc, 2006; Huang, Coull, 2008; Deligeorgiev et al., 2010; Shim, 2009; Jedrzejewska, 2010; Mishra, 2000; Vasilev, 2008; Shindy, 2015; Li et al., 1998) have a number of good properties, such as ease of synthesis, they are fluorescent, have higher photostability than the classical cyanine dyes and they can cover the spectrum from the UV to near infrared (NIR) region. So, in this manuscript we prepared different series of hemicyanine and aza-hemicyanine dyes as new synthesis contribution, spectroscopic investigation and antimicriobial evaluation in this field to may be used and/or applied in any of the wide distributed multidisciplinary uses and application of cyanine dyes, and particularly as sensitizers in manufacturing technology of photosensitive material industry and/or as batericidals in pharmacology and pharmaceutical industry.

2. Results and discussion

2.1. Synthesis:

Reaction of 3-ethyl-4-methyl-6-oxo-2-phenyl-furo[(3,2-d) pyrazolium, ($\bar{3},\bar{2}$– d) imidazole] iodide salts (1) (Shindy et al, 2016) with aromatic aldehyde (benzaldehyde, p.OH benzaldehyde, p.OCH_3 benzaldehyde, p.$N(CH_3)_2$ benzaldehyde, p.NO_2 benzaldehyde, p.Cl. benzaldehyde) in equimolar ratios, in ethanol as organic solvent and piperidine as a basic catalyst achieved the 4(1)-hemicyanine dyes (2a-f), Scheme (1), Table 1.

Equimolar reaction of (1) and the nitroso compounds (p-nitroso-phenol, α-nitroso-β-phenyl, β-nitroso-α-naphthol) in ethanol containing few mls of piperidine resulted the 4[2(1)]-aza-hemicyanine dyes (3a-c), Scheme (1), Table 1.

The structure of the prepared compounds was confirmed by elemental analysis, Table 1, visible spectra, Table 1, IR (Wade, 1999) and ^{1}H NMR (Wade, 1999) spectroscopic data, Table 2.

2.2. Spectral investigation:

The electronic visible absorption spectral of the hemicyanine dyes (2a-f) in 95 % ethanol solution gives bands in visible region 400-430 nm. These bands underwent displacements to give bathochromic shifts (red shifts) and/or hypsochromic shifts (blue shifts) in addition to increasing and/or decreasing the intensity of the absorption bands depending upon the type of the X substituents in the phenyl ring system, Scheme (1), Table 1.

So, substituting X = H in dye (2a) by X = OH, OCH_3 and/or $N(CH_3)_2$ to obtain dyes (2b), (2c) and/or (2d) makes bathochromic shifts for the absorption bands by 5 nm, 10 nm, 15 nm, in addition to increasing for the intensity of the bands, respectively, Scheme (1), Table 1. This can be attributed to the electron pushing characters of the OH, OCH_3 and/or $N(CH_3)_2$ groups in the latter dyes, which increase and/or facilitate the intensity of the electronic charge transfer to the quaternary nitrogen atom of the pyrazolium iodide salt (acidic center of the dye) and consequently red shifts occurs for the bands of these latter dyes (2b), (2c), (2d) in correspondence to the former parent dye (2a). Substituting X = H by X= NO_2 and/or Cl moving from dye (2a) to dyes (2e) and/or (2f) causes blue shifts for the absorption bands by 5 nm and/or 15, accompanied by quenching the intensity of these bands, respectively, Scheme (1), Table 1. This can be related to the strong electron pulling characters of the NO_2 group and/or the Cl atom in latter dyes (2e) and/or (2f) which make decreasing for intensity of electronic charge transfer to the quaternary nitrogen of the pyrazolium salt residue (acidic center of the dye), and accordingly hypsochromic shifts occurs in the spectra of the latter dyes (2e) and/or (2f) in correspondence to the parent dye (2a).

Additionally, the electronic visible absorption spectra of the aza-hemicyanine dyes (3a-c) in 95% ethanol solution reveals bands in the visible region 405-420 nm. The positions of these bands and their molar extinction coefficients are largely effected by the type of the phenyl and/or the naphthyl ring system in the dyes molecules, Scheme (1), Table (1).

So, substituting the benzene ring system in dye (3a) by naphthyl ring system to give dyes (3b) and/or (3c) resulted in a noticeable bathochromic shifts for the absorption bands by 9 nm and/or 15 nm in addition to increasing the intensity of the bands, respectively. This can be attributed to increasing π-delocalization conjugation the latter dyes (3b), (3c) due to the presence of naphthyl ring systems in correspondence to phenyl ring system in the former dye (3a).

It is also, interested to notice that, substituted by X = 2.OH, 5, 6-benz by X = 2.OH, 3,4-benz transferring form dye (3b) to dye (3c) cause bathochromic shifts for the absorption bands by 5 nm,

Scheme (1) Table (1). This may be related to the higher planarity of the dye (3c) in correspondence to the lower planarity of the dye (3b).

2.3. Antimicrobial evaluation:

Structural antimicrobial activity relationship for the hemicyanine dyes (2a-f) and the aza-hemicyanine dyes (3a-c) were studied, determined and evaluated against some bacterial and fungal strains (Escherichia coli, Staphylococcus aureus, Aspergillus flavus and Candida albicans), Table (3). From this study it was observed that:

The antimicrobial activity of the hemicyanine dyes (2a-e) undergo to give higher and/or lower inhibition zone diameter against the bacterial strains depending upon the type of the X substituents in the benzene ring of the aromatic aldehyde consisting the dyes structures, Table (3).

So, substituting X = H in dye (2a) by X = p.OH, p.OCH_3, p.$N(CH_3)_2$ and p.NO_2 to get dyes (2b), (2c), (2d), and (2e) makes lowering for the bacterial inhibition zone diameter against the bacterial strains, Table (3). This may be attributed to the presence of electron donating groups (p.OH, p.OCH_3, p.$N(CH_3)$ and/or the presence of electron attracting group (p.NO_2) the latter dyes, respectively, Table 3.

Comparison the antibacterial activity of the dye (2f) X= Cl by their analogous dyes (2b, X = p.OH), (2d, X = p.$N(CH_3)_2$, (2e, X = p.NO_2) showed that the former dye (2f) have higher biological activity against all the bacterial strains, Table (3). This may be related to the strong electron attracting character of the chlorine atom in the former dye (2f).

Also, it is noticed that, the antibacterial activity of the hemicyanine dye (2c, X = p.OCH_3) have higher antimicrobial activity if compared with their analogous dyes (2d, X = p.$N(CH_3)_2$) and (2e, X = p.NO_2), Table (3), This may be attributed to the oxygenated methyl group in the former dye (2c).

Comparison the antibacterial activity of the hemicyanine dyes (2a-f) showed that the dye (2a, X = H) gives the highest inhibition zone diameter against all the bacterial strains, Table (3). This reflects its increased ability to may used and/or applied as antimicrobial against these bacterial strains.

The comparison of the antimicrobial activity of the hemicyanine dyes (2a-f) declared that, the dye (2d, X = p.$N(CH_3)_2$) gives the lowest inhibition effect against all the bacterial strains, Table (3). This reflects its deficiency to may be used and/or applied as antibacterial active against these bacterial strains.

Comparing the antimicrobial activity of all the hemicyanine dyes (2a-f) declared that these compounds possesses higher inhibition effect against Escherichia coli bacterial strain compared with staphylococcus aureus bacterial strain, Table (3). This reflects their increased ability to may be used and/or applied as antibacterial against the former bacterial strain.

All the hemicyanine dyes (2a-f) do not have antifungal activity on the tested microorganisms (Aspergillus flavus and Candida albicans) where they give zero inhibition zone diameter potency against these fungal strains, Table (3). This reflects their complete deficiency and their negative effect to may be used and/or applied as antifungal against these fungal strains.

In addition, the antibacterial activity of the aza-hemicyanine dyes (3a-c) undergo to give higher and/or lower inhibition zone diameter against the bacterial strains depending upon the types of the nitroso compounds consisting the dyes structure. So, substituting X = 4.OH in the dye (3a) by X = 2.OH, 3,4-benz and/or 2.OH, 5,6-benz to get dyes (3b) and/or (3c) caused increasing for the antibacterial action against the bacterial strains, Table (3). This may be attributed to increasing conjugation in the latter (3b) and (3c) dyes due to the presence of the naphthyl ring system in corresponding to phenyl ring system in the former dye (3a).

Comparison the antimicrobial activity of the aza-hemicyanine dye (3b) with their analogues dye (3c) showed that the latter dye (3c) have higher potency diameter towards staphylococcus aureas bacterial strain than the former dye (3b), Table (3). This reflect its increased ability to be used and/or applied as antibacterial against this bacterial strains. This effect may be related to the higher planarity of this dye (3c) compared to the lower planarity of dye (3c).

The antimicrobial activity action of all the aza-hemicyanine dyes (3a-c) showed zero inhibition diameters against the fungal strains, Table (3). This reflects their negative effects and their complete deficiency to be used and/or applied as antimicrobial against these fungal strains.

Replacing the dimethine group (CH=CH) in the hemicyanine dye (2b) by the azamethane group (CH=N) to get the aza-hemicyanine dye (3a) makes increasing for the antimicrobial activity against the staphylococcus aureas bacterial strain, Table (3). This may be related to the effect of the azamethane group (CH=N) in the latter dye (3a).

Comparison the antimicrobial activity of the hemicyanine dyes (2a-f) and the aza-hemicyanine dyes (3a-c) declared that the latter aza-hemicyanine dyes (3a-c) are higher biological active compounds than the former hemicyanine dyes (2a-f) against the bacterial strains, Table (3). This may be related to the presence of the azamethane group (-CH=N-) in the latter aza-hemicyanine dyes (3a-c) in correspondence to the dimethine group (-CH=CH-) in the former hemicyanine dyes (3a-f).

General cmparison the antimicrobial effects of the tested compounds showed that the aza-hemicyanine dye (3c) gives the highest inhibitor zone diameter against the bacterial strains, Table (3). This reflects its increased effects and/or its higher availability to may be used and/or applied as antimicrobial against these bacterial strains. In contrast the hemicyanine dye (2d) gives the the lowest inhibition zone diameter against the bacterial strains, Table (3). This indicates its decreased effects and/or its lower availability to may be used and/or applied as antimicrobial against these bacterial strains.

3. Conclusion

Following are major conclusions were drawn from this study:

1. The electronic visible absorption spectra of hemicyanine dyes and/or the aza-hemicyanine dyes in 95 % ethanol solution underwent displacements to give bathochromic and/or hypsochromic band shifts in addition to increasing and/or decreasing the intensity of the absorption bands depending upon the following factors:

a) Presence of electron releasing and/or attracting groups in the dyes molecules in the order of: electron pushing group dyes > electron pulling group dyes (for the hemicyanine dye).

b) Presence of phenyl and/or naphthyl ring system in the order of: naphthyl dyes > phenyl dyes (for aza-hemicyanine dyes).

c) Planarity of the dyes in the order of: higher planarity dyes > lower planarity dyes (for aza-hemicyanine dyes).

2. The intensity of the colours of the hemicyanine dyes and/or the aza-hemicyanine dyes can be related to the two suggested mesomeric structures (A) and (B) producing a delocalized positive charge over the conjugated system, Scheme (2).

3. The antimicrobial inhibition zone diameters of the tested hemicyanine dyes (2a-f) and the aza-hemicyanine dyes (3a-c) underwent to give higher and/or lower inhibition potency depending upon the following factors:

a) Type of the X substituted in the aromatic aldehyde consisting the dyes structures for the hemicyanine dyes (2a-f) in the order of:

i) p.H dye > p.OH dye > p.$N(CH_3)_2$ dye.

ii) p.OCH_3 dye > p.NO_2 dye > p.OH dye > p.$N(CH_3)_2$ dye.

iii) p.Cl dye > p.NO_2 dye > p.OH dye > p.$N(CH_3)_2$ dye.

b) Presence of phenyl and/or naphthyl ring system for aza-hemicyanine dyes in the order of: naphthyl dyes > phenyl dyes (2.OH, 5,6-benz dye > 2.OH, 3,4-benz dye > 4.OH dye)

c) Planarity of the dyes for aza-hemicyanine dyes in the order of: higher planarity dyes > lower planarity dyes (2.OH, 5,6-benz dye > 2.OH, 3,4-benz dye).

d) Kinds of the bacterial strains in the order of: higher in the case of Escherichia coloi bacterial strain compared to the staphylococcus aureas bacterial strain.

e) Bacterial and/or fungal strains in the order of: most samples have antibacterial activity, but all of them do not have any antifungal activity.

4. Experimental

4.1. General:

All the melting points of the prepared compounds are measured using Electrothermal 15V, 45W 1 A9100 melting point apparatus, Chemistry department, Faculty of Science (Aswan University) and are uncorrected. Elemental analysis were carried out at the Microanalytical Center of Cairo University by an automatic analyzer (Vario EL III Germany). Infrared spectra were

measured with a FT/IR (4100 Jasco Japan), Cairo University. ^{1}H NMR Spectra were accomplished using Varian Gemini-300 MHz NMR Spectrometer (Cairo University). Electronic visible absorption spectra were carried out on Visible Spectrophotometer, Spectro 24 RS Labomed, INC, Chemistry department, Faculty of Science (Aswan University). Antimicrobial activity was carried out at the Microanalytical center, Microbiology division (Cairo University).

4.2. Synthesis:

4.2.1. Synthesis of 3–ethyl–6–oxo–2–phenyl–furo [3,2–d) ptrazole, (3,2–d) imidazole]-4-(1)] hemicyanine dyes (2a-f).

Quaternized compound (1) (0.01 mol) and equimolar ratios of (benzaldehyde-4-hydroxy benzaldehyde, 4-methoxy benzaldehyde, p.N,N-dimethylaminobenzaldehyde, 4-nitrobenzaldehyde or 4-chlorobenzaldehyde) were refluxed in ethanol (20-30 ml) as solvent containing piperidine (1-2 ml) as catalyst for about 6 hrs. The reaction mixture changed from reddish color to dark brown at the end of the refluxing. It was filtered while hot to remove unreacted materials, cooled and precipitated in ice-water mixture. The hemicyanine dyes (2a-f) were collected, washed with water several times, dried and crystallized from ethanol, Table (1).

4.2.2. Synthesis of 3–ethyl–6–oxo–2–phenyl–furo [3,2–d) ptrazole, (3,2–d) imidazole]-4-(1)] aza-hemicyanine dyes (3a-c).

The quaternized compound (1) (0.01 mol) and equimolar ratios of either 4-nitroso phenol, 1-nitroso-2-naphthol; or 2-nitroso-1-naphthol in ethanol (30 ml)containing piperidine (1-2 ml) were heated under refluxed for 6 hrs. The reaction mixture which attained a deep permanent colour at the end of refluxing was filtered on hot to remove any impurities precipitated using ice-water mixture and dried. The aza-hemicyanine dyes (3a-c) were collected and crystallized using ethanol. See the data given in Table (1).

4.3. Spectral investigation:

The electronic visible absorption spectra of the prepared cyanine dyes were examined in 95 % ethanol solution and recorded using 1 cm Q_z cell in Visible Spectrophotometer, Spectro 24 RS Labomed, INC. A stock solution (1 x 10^{-3}M) of the dyes was prepared and diluted to a suitable volume in order to obtain the desired lower concentrations. The spectra were recorded immediately to eliminate as much as possible the effect of time.

4.4. Antimicrobial evaluation:

The tested compounds (2a, 2b, 2c, 2d, 2e,2f 3a, 3b, 3c) were dissolved in DMSO to give a final concentration (l mgm/ml). Susceptible sterile discs were impregnated by the tested substance (50 µgm/disc) via a means of micropipette. The biological activity for each substance was tested on surface -seeded nutrient agar medium with the prepared susceptible discs, Bacterial strains and the biological effect are shown in Table 3.

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References

Kim, 2006 – *Kim S.H.* (2006). Functional Dyes, Oxford: Elsevier B. V., 47-84.

Hammer, 1964 – *Hammer F.* (1964). Cyanine Dyes and Related Compounds (London/New York: Interscience Publisher, 1964). 398.

Raue, 1990 – *Raue R.* (1990). In Ullmann's Encyclopedia of Industrial Chemistry, Vol. 16, Eds BElver: SHawkins and GShuiz (Weinheim: Wiley VCH Verlag, 1990) 427.

Pardal et al., 2002 – *Pardal C., Ramos S.S., Santos P.F., Reis L.V., Almeida P.* (2002). Synthesis and spectroscopic characterization N-alkyl quaternary ammonium salts typical precursors of cyanines, Molecules, 7, 320.

Vassilev, 2004 – *Vassilev A., Dikova I., Deligeorgiev T. and K-H. Drexhage* (2004). Quaternization of N-heterocyles with propylene oxide and epichlorohydrin, *Synth. Commun.*, 34, 2539.

Deligeorgiev, 2005 – *Deligeorgiev T., Vassilev A. and Drexhage K-H.* (2005). Synthesis of novel intermediates for cyanine dyes by the quaternization of N-heterocycles with acylamide and N-alkyl acylamides, *Dyes Pigm.*, 67, 21.

Mazinres, 2007 – *Mazinres M.R., Duprat C., Bellan J. and Wolf J.G.* (2007). Synthesis and characterization of new phosphonate labeled cyanines, *Dyes Pigm.*, 74, 404.

Nagarajan, Perumal, 2004 – Nagarajan R., Perumal P.T. (2004). A facil methode for the synthesis of acetylcarbazoles and carbazole aldehydes, *Synth. Commun.*, 34, 2127.

Lai et al., 1997 – Lai G., Bu X.R., Santos J., Mintz E.A. (1997). Reinvestigation of the vilsmeier-Haack formylation of triphenylamine. *Synlett*; 11, 1275.

Kabatc et al., 2015 – *Kabatc J., Jurek K., Kostrzewska K., Orzel L.* (2015). Hemicyanine dyes derived from 2,3,3-trimethyl-3H-indolium as candidates for non-covalent protein probes, *J. Pharm. Biomed. Anal.*, 114, 433-440.

Malegoll et al., 2005 – *Malegoll T., Gmouh S., Meziane M.A.A., Blanchard-Desce M., Mongin O.* (2005). Practical and efficient synthesis of tris(4-formylphenyl) amine, a key building block in materials chemistry. *Synthesis*, 11, 1771.

Narayan, Ansari, 2008 – *Narayan B., Ansari S.* (2008). Synthesis and spectroscopic studies of some butadienyl chain substituted benzothiazole asycyanine colorants. *Oriental Journal of Chemistry*, 24 (3), 1115-1118.

Davis et al., 2004 – *Davis M.C., Chafin A.P., Hollins R.A., Ealdwin L.C., Derickson E., Zarras P., Drury E.* (2004). Synthesis of an isophorone-based nonlinear optical chromophore, Synth. Commun., 34, 3419.

Shindy, Koraiem, 2008 – *Shindy H.A., Koraiem A.I.M.* (2008). Synthesis and colour spectroscopic investigation of some hemicyanine dyes. Color. Technol. 124 (5), 290-294.

Shindy et al., 2014 – *Shindy H.A., El-Maghraby M.A., Eissa F.M.* (2014). Synthesis and spectral properties of novel hemicyanine dyes, Russ. Chem. Bull. [Izv. AN. Ser. Khim.], 707-715 (in Russian), Russ. Chem. Bull., Int. Ed. 2014, 63 (3), 707-715.

Davis et al., 2006 – *Davis M.C., Hollins R.A., Douglas B.* (2006). Synthesis of thiophene-based nonlinear optical chromophore, *Synth. Commun.*, 36, 3515.

Wuskell et al., 2006 – *Wuskell J.P., Boudreau D., Wei M., Jin L., Engl R., Chebolu R., Bullen A., Hoffacker K.D., Kerimo J., Cohen L.B., Zochowski M.R., Loew L.M.* Synthesis, spectra, delivery and potentiometric responses of new styryl dyes with extend spectral ranges, J Neurosci. *Methods*, 151 (2006) 200.

Banerji et al., 1982 – *Banerji J.C., Mandal A.K., Banerje B.K.* (1982). Methine chain substituted styryl cyanine dyes-part I, *Dyes Pigm.*, 3, 273.

Jha, Banerji, 1985 – *Jha B.N., Banerji J.C.* (1985). Chromophoric chain B-aryl-substituted styryl cyanines: effect of substituents on visible absorption spectra and photosensitization properties, *Dyes Pigm.*, 6, 213.

Jha, 1986 – *Jha B.N., Jha R.K., Banerji J.C.* (1986). Chromophoric chain B-(styryl, substituted styryl and phenyl-substituted butadienyl) cyanines: effect of B-substituents and chain lengthening, *Dyes Pigm.*, 7, 133.

Heilbron, Walter, 1925 – *Heilbron K., Walter B.* (1925). Styryl benzopyrilium salts. Part V. Distyryl derivatives of 7-hydroxy-2:4-dimethyl benzopyrilium chloride, *J Chem. Soc.*, 127 (1925) 690.

Ren et al., 2012 – Ren Z., Qin C., Tang R., Chen C. (2012). Study on the dyeing properties of hemicyanine dyes. II. Cationic dyeable polyester, Color. Technol., 128, 147-152.

Shindy, 2015 – *Shindy H.A.* (2015). Synthesis of different classes of benz (naphth) / five membered heterocyclic cyanine dyes: *A Review, Rev. Roum. Chim.*, 60 (1), 5-13.

Shindy, 2007 –*Shindy H.A.* (2007). Synthesis, characterization and visible spectral behaviour of some novel methine, styryl and aza-styryl cyanine dyes, *Dyes and Pigments*, 75, 344-350.

Huang et al, 2002 – *Huang Y., Cheng T., Li F., Huang C.* (2002). Photophysical studies on the mono- and dichromophoric hemicyanine dyes I. Photoelectric conversion from the dye modified ITO electrodes, *J. Phys. Chem. B.*, 106, 10020-10030.

Antonious, 1997 – *Antonious M.S.* (1997). Solvent polarity indicators: flexible styryl pyridinium and quinolinium fluorescence probes for medium free-volume, *Spectrochim. Aca A,* 53 (11) 1997, 317.

Kabatc, 2006 – *Kabatc J., Osmidowski B., Paczkowski J.* (2006). The experimental studies on the determination of the ground and excited state dipole moments of some hemicyanine dyes. *Spectrochimica Acta Part A: Molecular and Biomolecular spectroscopy*, 63 (3), 524-531.

Huang, Coull, 2008 – *Huang Y., Coull J.M.* (2008). Diamine catalyzed hemicyanine dye formation from non fluorescent precursors through DNA programmed chemistry, J. Am. Chem. Soc., 130 (11), 3238-3239

Deligeorgiev et al., 2010 – *Deligeorgiev T., Vasilev A., Kaloyanova S., Vaquero J.J.* (2010). Styryl dyes-synthesis and applications during the last 15 years, *Color. Technol.* 126 (2), 55-80.

Shim, 2009 – *Shim T., Lee M.H., Kim D., Kim H.S., Yoon K.B.* (2009). Fluorescence properties of hemicyanine in the nanoporous materials with varying pore sizes, *J. Phys. Chem. B*, 113 (4), 966-969.

Jedrzejewska, 2010 – *Jedrzejewska B., Pietrzak M., Paczkowski J.* (2010). Solvent effectson the spectroscopic properties of styryl quinolinium dyes series, *Journal of fluorescence*, 2010, 20 (1), 73-86.

Mishra, 2000 – *Mishra A., Behera R.K., Behera P.K., Mishra B.K., Behera G.B.* (2000). Cyanines during the 1990s: A Review, *Chem. Rev.*, 100, 1973.

Vasilev, 2008 – *Vasilev A., Deligeorgiev T., Gadjev N., Kaloyanova S., Vaquero J.J., Alvarez-Buil J., Baeza A.G.* (2008). Novel environmentally bengin procedures for the synthesis of styryl dyes, *Dyes Pigm.,* 77, 550.

Shindy, 2015 – *Shindy H.A.* (2015). Different methods in the synthesis of mono(bi)-heterocyclic six membered cyanine dyes: A Review, Organic Chemistry: *An Indian Journal*, 11 (1), 26-36.

Li et al., 1998 – *Li Q., Lin G-L., Peng B-X., ZH-x Li.* (1998). Synthesis, characterization and photographic properties of some new styryl cyanine dyes, *Dyes Pigm.*, 38, 211.

Jaung et al., 1996 – Jaung J.Y., Matsuoka M., Fukunishi K. (1996). Synthesis and properties of new styryl dyes derived from 2,3-dicyano-5-methyl pyrazines, *Dyes Pigm.,* 31, 141.

Shindy, 2016 – *Shindy H.A., Khalafalla A.K., Goma M.M., A.H. Eed* (2016). Polyheterocyclic compound in the synthesis and spectral studies of some novel methine cyanine dyes, *Revue Roumaine de Chimie*, 61 (3), 139-145.

Wade, 1999 – *Wade Jr.* (1999). L.G., Organic. Chemistry., 4th Edn (New Jersy Printice Hall, 500.

Wade, 1999 – *Wade L.G.* (1999). Organic. Chemistry., 4th Edn (New Jersy Printice Hall, 1999) 544.

Table 1. Characterization of the prepared compounds (2a-f), (3a-c).

Comp. No.	Nature of products			Molecular formula (M.Wt.)	Analysis%						Absorption spectra in 95% ethanol	
					Calculated			Found				
	Colour	yield %	M.P. C°		C	H	N	C	H	N	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^2$)
2a	Brown	61	130	$C_{22}H_{19}N_4O_2I$ (498)	53.01	3.81	11.24	52.95	3.72	11.12	415	17000

2b	Deep brown	50	120	$C_{22}H_{19}N_4O_3I$ (514)	51.36	3.69	10.89	51.21	3.60	10.77	420	35000
2c	Deep brown	45	145	$C_{23}H_{21}N_4O_3I$ (528)	52.27	3.97	10.60	52.16	3.80	10.53	425	24000
2d	Deep brown	43	95	$C_{24}H_{24}N_5O_2I$ (541)	53.23	4.43	12.93	53.20	4.32	12.79	430	23000
2e	Brown	54	135	$C_{22}H_{18}N_5O_4I$ (543)	48.61	3.31	12.89	48.50	3.12	12.70	410	21000
2f	Brown	40	120	C22H18N4O2ClI (533)	49.57	3.38	10.51	49.44	3.29	10.46	400	35000
3a	Pale brown	55	100	C21H18N5O3I (515)	48.93	3.49	13.59	48.87	3.38	13.45	405	25000
3b	Deep brown	55	125	C25H20N5O3I (565)	53.09	3.53	12.38	53.00	3.42	12.13	414	57700
3c	Deep brown	60	155	C25H20N5O3I (565)	53.09	3.53	12.38	52.88	3.41	12.31	420	40000

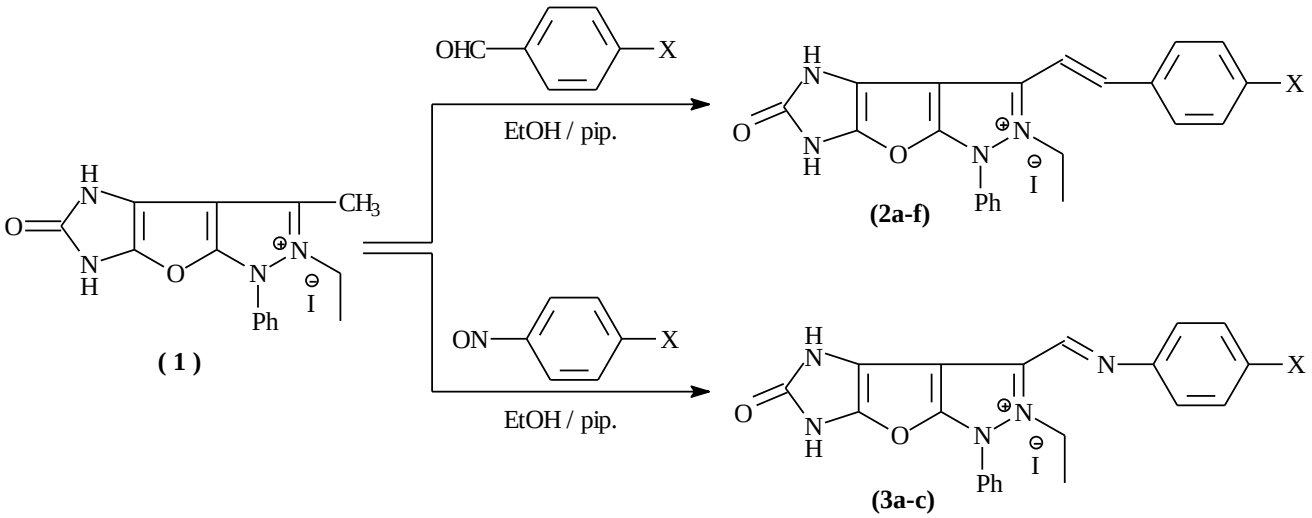
Table 2. IR and ^{1}H NMR spectral data of the prepared compounds

Comp. No	IR Spectrum (KBr, Cm^{-1})	^{1}H NMR Spectrum (DMSO, δ)
2a	691, 648(monosubstituted phenyl). 1025, 1065, 1118, 1164(C-O-C cyclic). 1552, 1496 (C=N). 1597 (C=C). 1715 (C=O). 2924, 2854 (quaternary salt). 3417 (NH).	1.2-1.6 (m, 3H, CH_3 of position 3). 3.3 (b, 2H, CH_2 of position 3). 7.1 (b, 2H, 2NH). 7.3-8.2(m, 12H, aromatic + 2 -CH=).
2b	642, 692(monosubstituted phenyl). 759, 888 (p.disubstituted phenyl). 1152 (C-O-C cyclic). 1496 (C=N).	1.2-1.7 (m, 3H, CH_3 of position 3). 3.4 (b, 2H, CH_2 of position 3). 6.9 (s, 1H, OH). 7.3 (b, 2H, 2NH).

	1594 (C=C). 1712 (C=O). 2939, 2856 (quaternary salt). 3414 (NH).	7.4-8.2(m, 11H, aromatic + 2 -CH=).
3a	644, 692 (monosubstituted phenyl). 757, 832 (p.disubstituted phenyl). 1065, 1123, 1163(C-O-C cyclic). 1496, 1546 (C=N). 1597 (C=C). 1714 (C=O). 2933, 2855 (quaternary salt). 3059 (OH). 3420 (NH).	1.3-1.8 (m, 3H, CH_3 of position 3). 3.4 (b, 2H, CH_2 of position 3). 6.9 (b, 1H, OH). 7.2 (b, 2H, 2NH). 7.3-8.4(m, 10H, aromatic+ -CH=).

Table 3. The antimicrobial activity of compounds 2a-f, 3a-c.

Sample	**Inhibition zone diameter (mm/mg sample)**			
	Escherichia coli **(G^-)**	***Staphylococcus aureus*** **(G^+)**	***Aspergillus flavus*** **(Fungus)**	***Candida albicans*** **(Fungus)**
Control DMSO	0.0	0.0	0.0	0.0
2a	12	11	0.0	0.0
2b	10	0.0	0.0	0.0
2c	12	10	0.0	0.0
2d	9	0.0	0.0	0.0
2e	10	9	0.0	0.0
2f	12	10	0.0	0.0
3a	10	11	0.0	0.0
3b	15	15	0.0	0.0
3c	15	17	0.0	0.0



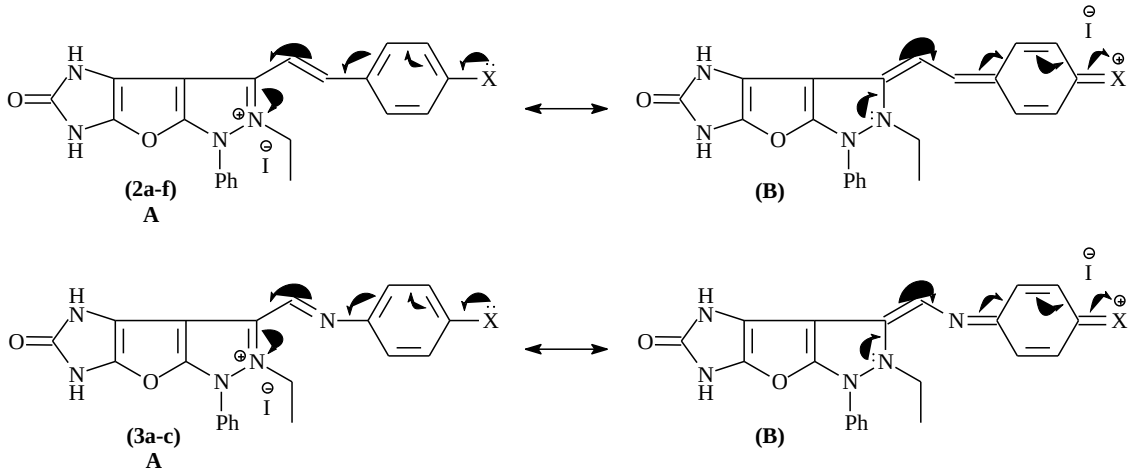
Synthesis routes of the prepared hemicyanine (2a-f) and aza-hemicyanine (3a-c) dyes

Scheme (1)

<u>Substituents in Scheme (1)</u>:

(2a-f): X = H (a); OH (b), OCH_3 (c), $N(CH_3)_2$ (d), NO_2 (e), Cl (f).

(3a-c): X = 4.OH (a), 2.OH; 3,4-benzo (b); 2.OH, 5,6-benzo (c).



Colour intensity illustration of the synthesized hemicyanine (2a-f) and aza-hemicyanine (3a-c) dyes

Scheme (2)

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Tissue Engineering Constructs for Osteoarthritis Treatment: a Control of Remodeling

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Abstract

Tissue engineering and regenerative medicine technologies (TERM technologies) remarkable progress allowed to use predominantly minimally invasive arthroscopic techniques to treat traumas and chronic joint diseases. The essence of joints tissue engineering is development and manufacturing bioengineering matrices (scaffolds) and their following implantation in cell-free variant, or previously populated by suitable cell pool to recover defects by a full-value 3D-structure. The important challenge here is to make individualized scaffolds, which properties are meet the requirements of person and his cartilage defect. The main objective of the study is to describe tissue engineering system "cartilage – scaffold" using systems biology and biocybernetics approaches. The task is to predict development of considered system through time and investigate the possibility to define and solve the control problem which could open the door for property-oriented scaffolds development. Authors explored the referred tissue engineering system as feedback-controlled system. Then we proposed the system of difference equations, which describe its dynamics. Results of computer simulation and forecasted values of extracellular matrix and cells volume ration are in physiological intervals and on a first approximation correspond with previously obtained experimental ones. The next step is to modify model for inverse solution of developing new generation of tissue engineering implants with predefined and controlled characteristics.

Keywords: tissue engineering, regenerative medicine, articular cartilage, osteoarthritis, biological systems, simulation modeling.

1. Introduction

Tissue engineering and regenerative medicine technologies (TERM technologies) progress in articular cartilage repair determines by well-defined complex of social, economic, medical and biological factors, Firstly, lifespan at developed countries steady increase with the proportion of senior citizens lead an active life. As consequence, joint decease prevalence and demand of high (movement capability in multilevel buildings, car driving, farmland works, tourist trips, etc.) quality of life growth simultaneously. In addition to degenerative changes in joint, specialists point to big joints traumas increase because of technology expansion in all fields of life and, also, extremism (Hunziker, 2009; van Osch et al., 2009). Secondly, remarkable progress of materials and medical technology allowed to use predominantly minimally invasive arthroscopic techniques to treat traumas and chronic joint diseases. At least, the object of treatment – articular cartilage –

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has low regenerative capability, thus in most cases one needs not only to compensate lost structures of articular cartilage and its functions but also to stimulate patient's own cells to remodel zone of cartilage defect replacement into a proper cartilage tissue. (de Isla et al., 2010; Mao, 2015)

The primary function of joint is locomotion – a complex of synchronized motions enables an organism to move. The key component of joint to sustain this function is hyaline cartilage. Low coefficient of contact friction in flexible joint is needed for movements coordination. Such condition is provided by cartilage and synovial liquid, which figures as lubricant. Also, cartilage damps and redistributes loads to keep the subjacent bone intact (McNary et al., 2012; Giorgi et al., 2016).

So, the goal of cartilage as biological system is to reach the state, when: the coefficient of contact friction is in physiological interval (1), and physiological loads damping and redistributing are successful (2). To achieve this goal, the control of cartilage maintains at two levels - central and local ones. The central nervous system controls cartilage through using signals from joint and skin mechanoreceptors. When they signaling the joint malfunction and destruction threat (one suffer pain, for example), organism starts to avoid painful movements. In some cases, one could ignore such signals within certain limitations – when training, for instance. As result, articular cartilage structure will change at the cell and molecular level. Processes at this level are exactly defined the actual joint characteristics and its abilities. That is why we will discuss processes' control at this level only.

The current "gold standard" technique of damaged cartilage recovery is autogenous chondroplasty. This classical approach has several intractable limitations, disadvantages and eventually does not provide adequate restoration of joint function for a long time. Most of specialists in regenerative medicine consider tissue engineering technologies to be a next leader in this area (Getgood et al., 2009; Lu et al., 2013).

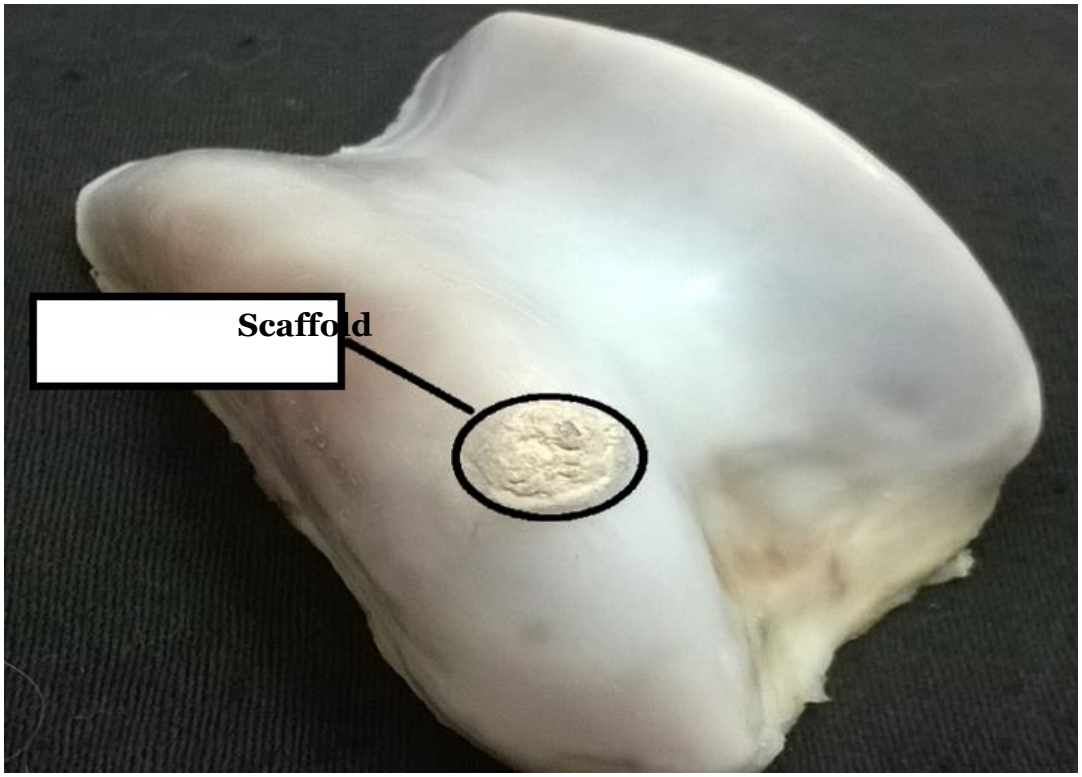

Fig. 1. Chitosan scaffold on bovine cartilage before remodeling differ from surrounding cartilage in its structure and properties.

The essence of joints tissue engineering is development and manufacturing bioengineering matrices (scaffolds) and their following implantation in cell-free variant, or previously populated by suitable cell pool to recover defect and to stimulate damaged tissue 3D-structure. The key

problem is to completely remodel tissue engineering construction to native cartilage. The solution needs to use predictable control of cells population, proliferation, differentiation and adequate phenotypical expression in scaffold's material and future native cartilage matrix. One of key approaches to such of control is planning and manufacturing scaffold with predefined complex of its properties (O'Brien, 2011; Zohreh et al., 2012).

Currently there is a wide spectrum of materials suitable for making scaffolds. Such materials must meet the following prerequisites: cytotoxicity and inflammatory and immune response absence, support of cells adhesion, fixation, proliferation and differentiation, bioresorbtion through common metabolic pathways, ability of self-recovering, structure and characteristics changing in response to environmental factors, including physical stress (Zhang et al., 2009; Correia et al., 2011; Kuo, 2011; Bogatov et al., 2015; Maitz, 2015).

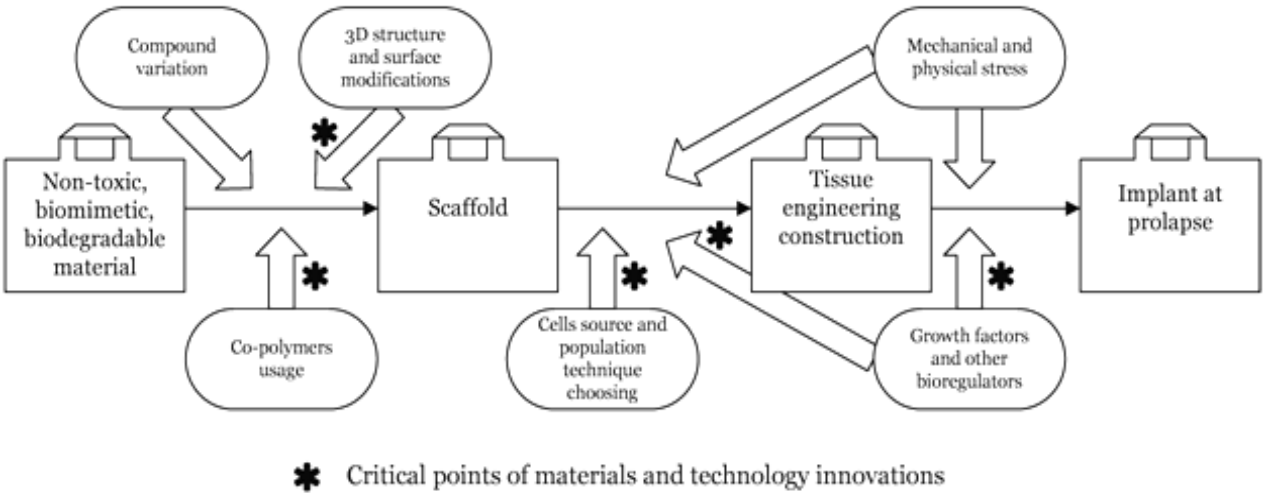

Fig. 2. The schema of crucial processes responsible to quality of scaffold-technologies for articular cartilage repair demonstrates critical point of material and technology innovation.

One of promising approaches applies high hydrostatic pressure (HHP) to treat cartilage for its repair. Cartilage devitalization using HHP demonstrates effective cellular deactivation when tissue structure remains intact. Then chondrocytes and mesenchymal stem cells are successfully cultured on devitalized cartilage (Hiemer et al., 2016).

High ability for chondrogenic differentiation of umbilical cord blood mesenchymal stem cells (UCB-MSCs) demonstrates at this (Gómez-Leduc et al., 2016) study. Authors combined 3D culture in type I/III collagen sponges and chondrogenic factors. Results showed that UCB-MSCs have a high proliferative capacity and that human ones can be a reliable source for cartilage tissue engineering.

The preceding study (Shiroky, 2014) explained the renewal of the articular cartilage in normalcy and osteoarthritis development by principles of mathematical modeling. Such models help to develop advanced methods of prevention, detection and treatment of osteoarthritis including molecular biotechnologies based on tissue engineering conception. We used histological images to perform structural analysis to discover the signs of active system and its states. Received data are useful to develop research protocols in cartilage tissue engineering.

Now the study continues to describe tissue engineering system 'cartilage – scaffold' using systems biology and biocybernetics approaches. The objective is to predict development of considered system through time and investigate the possibility to define and solve the control problem which could open the door for property-oriented scaffolds development.

2. Material and Methods

Role of scaffold' structure and biochemical properties in cartilage remodeling

Articular cartilage unable to regenerate when osteoarthritis (Hunziker, 2009). The common way to treat such a serious degenerative pathology is total articular replacement, which is horribly traumatic operation leads to long rehabilitation period.

A more prospective way is to use tissue engineering constructs – scaffolds. Scaffold is complex three-dimensional biomimetic implant made of customized biopolymer like native cartilage tissue in density and load damping and redistribution ability. And it is not a prosthesis – cartilage cells populate scaffold and then, during 8–12 weeks, remodel it into a native extracellular matrix (ECM). Thus, scaffold disappears leaving behind physiological healthy cartilage (Fitzpatrick, 2015; Ivanov et al., 2015). However, complete remodeling takes quite a long time.

An important characteristic of scaffold is its three-dimensional vesicular structure with specific size of pores and thickness of barriers between them. According to experimental research, suitable porosity for cartilage repair is about 80–85 % with pores diameter in range 150–400 mkm and barriers thickness not less than 50–70 mkm. It is necessary to provide the specific integrity, high cells adhesion ability and, simultaneously, possibility of gases and metabolites transport in newly originating tissues (da Silva et al., 2010; Bhardwaj et al., 2011; O'Brien, 2011). Currently researches focused on modifying scaffold-technologies by varying co-polymers, making nanostructured products and adding growth factors depot.

Growth factors and other biologically active substances supporting chondrocytes adhesion and proliferation are particularly important to make scaffolds for cartilage repair and remodeling (Elder, 2009; Novochadov, 2013; Almalki, 2016).

It is known that such powerful bioactive substance as insulin causes chondral differentiation. The study (Malafaya et al., 2010) is devoted to chondrogenous differentiation and growth stimulation of cell systems synthesizing biomolecules. For that purpose, various forms of insulin have been added to scaffold as potential model system of cartilage. Insulin dose of 5 % at the system was proven as the most effective to stimulate chondrogenous differentiation.

Therefore, scaffolds are continuously improving by adding growth factors (Novochadov, 2013) and other signal molecules, which stimulates cartilage tissue synthesis and accelerating the remodeling. Unfortunately, these molecules are expensive – therefore two conflicting problems are rising: to minimize the time of complete remodeling (1), and to reduce scaffold cost by minimizing concentrations of signal molecules (2).

There are two main approaches to solve these problems. The first one is to modify structure of scaffold by changing its porosity and three-dimensional configuration. The second approach is to manipulate attitude, concentration and activation time of signal molecules. We discuss the last one here.

The controlling object in considered tissue engineering system is the cell pool of chondrocytes which are remodel the cartilage. The controlling action is signal molecules – growth factors and cytokines (Goldring, 2012). The structural schema of the system is on Fig. 3.

Naturally this system is error-actuated. For example, matrix slowly scuffs when moving and its wear debris enters the synovia. This leads, on the first hand, to rising its viscosity, lowering the friction coefficient and, on the second hand, to activation of phagocytes, absorbing tissue shreds and emitting cytokines, which are accelerating the degradation of ECM (Zhen, 2014).

Definition of control problem

Let us set the $x = (M, C)$, where $M \in [0, 1]$ is volume of native ECM in remodeling zone and $C \in [0, 1]$ is volume of chondrocytes there, is a phase vector describing the system state. The initial state of system corresponds to point $x_o = (M_0, C_0)$, where M_0=0 directly after the implantation (native ECM is absent), and C_0=0,01 (some chondrocytes infiltrate into the implant instantly and their volume estimate is 1 %).

Biologically reasonable constraints to phase variables is:

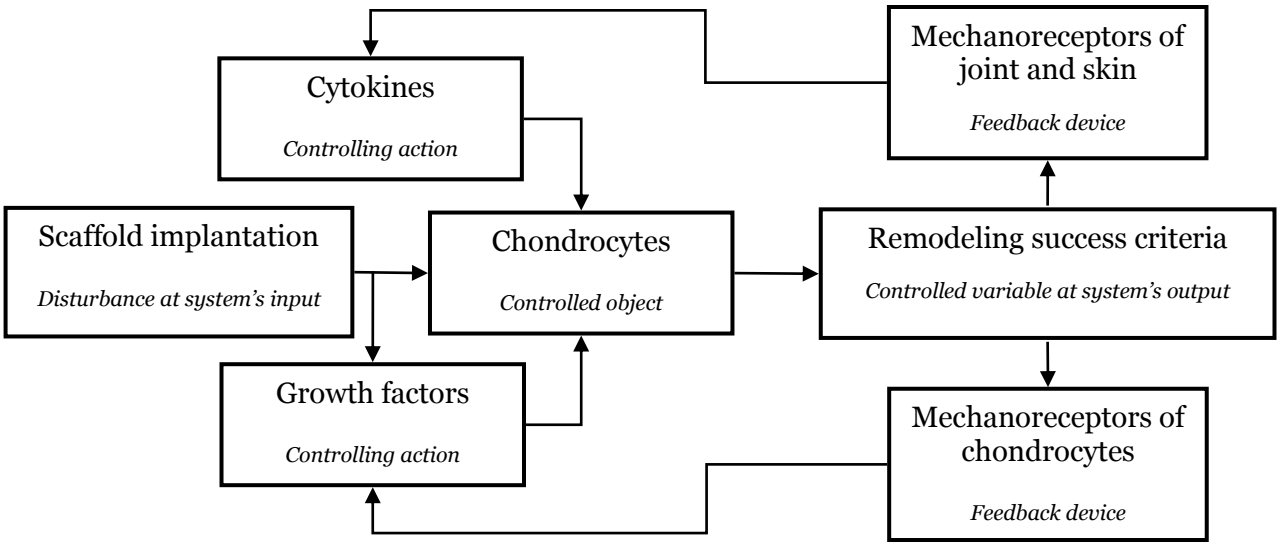

Fig. 3. The structure of the 'Cartilage – Scaffold' controlled system, its components and biological counterparts

$$(1)\ \begin{cases} 0 \le M \le 0{,}92; \\ 0{,}01 \le C \le 0{,}12; \\ M + C \le 1. \end{cases}$$

Values at the right part of inequalities were obtained during the numerous measurements of articular cartilage.

Implant could contain specific signal molecules with predefined spatial distribution and activation time. Consequently, the controlling action is a distribution and activation function of the specific molecule. In one-dimensional case it is written $u_i(l, t)$, where i is molecule's index, $l \in [0, 1]$ is a distance for scaffold's surface, t is time since the implantation. At that, if t_i^0 is the activations time of i-th molecule at point l_0, then when $t < t_i^0$ $u_i(l_0, t) = 0$, $u_i(l_0, t_i^0) = \max_t u_i(l_0, t)$, and when $t > t_i^0$ $u_i(l_0, t)$ is decreasing logarithmically to level of normal concentration.

Let us describe dependencies between coefficients of equations. Consider to system with four controls, corresponding to basic controlling molecules TGF-β, BMP-7, IL-1α/IL-1β, TNF-α. Table 1 contains description of their influence to biological processes in system.

Table 1. Mutual influence of signal molecules to corresponding biological processes flow rate

The control and basic signal molecule		$u_1(l, t)$ TGF-β	$u_2(l, t)$ BMP-7	$u_3(l, t)$ TNF-α	$u_4(l, t)$ IL-1α/IL-1β
Corresponding phase variable and influence direction		$C+$	$M+$	$C-$	$M-$
Additional molecules and their influence	TNF-α	+ +	– –		+ + +
	IL-1	+	–	+ +	

The X signal molecule when present in remodeling zone could influence to flow rate of process, started by Y molecule. At that the coefficient of X influence to Y usually lies in ranges: [1.5; 3.0) (at + / –), [3.0; 10.0) (at + + / – –), [10.0; 30.0] (at + + + / – – –).

Considering the Table 1 we obtain the following set of difference equations:

$$(2)\quad \begin{cases} M(t+\Delta t) = M(t)\left(k_2 u_2(l,t) k_3^2 u_3(l,t) k_4^2 u_4(l,t) - k_3^4 u_3(l,t) k_4 u_4(l,t)\right); \\ C(t+\Delta t) = C(t)\left(k_1 u_1(l,t) k_3^1 \frac{1}{u_3} k_4^1 \frac{1}{u_4} + k_3 u_3(l,t) k_4^3 u_4(l,t) \right). \end{cases}$$

3. Results and discussion

Simulation Results

Values of controls and coefficients of equations set (2) come from published and available for free measurements of growth factors and cytokines influence to vital activity of the cartilage. The article (Asanbaeva et al., 2008) contains made in controlled study design measurements of cells population and collagen amount in young growing cartilage at 0 and at 13-th day since starting simulation of various growth factors. The publication (Riera et al., 2011) contains evidence of pro-inflammatory cytokines influence to cartilage cells proliferation and differentiation.

Consider the simple case, when signal molecules and cartilage cells are uniformly distributed at remodeling zone. We also suppose the linear dependence between molecule concentration and their effect. Then the following rules occur (Table 2):

Table 2. Estimate controlling actions influence to phase variables values

Signal molecule	Concentration at the remodeling zone	Influence to phase variable in a time Δt = 1 week
None (natural growth/loss)	none	$C(t+\Delta t) = C(t) - 0{,}057 \times C(t)$ $M(t+\Delta t) = M(t) + 0{,}13 \times M(t)$
TGF-β	10 ng/ml	$C(t+\Delta t) = C(t) + 0{,}027 \times C(t)$
BMP-7	50 ng/ml	$M(t+\Delta t) = M(t) + 0{,}04 \times M(t)$
TNF-α	10 ng/ml	$C(t+\Delta t) = C(t) - 0{,}23 \times C(t)$
IL-1α/IL-1β	10 ng/ml	$M(t+\Delta t) = M(t) - 0{,}02 \times M(t)$

The Table 3 contains biologically rational values of additional signal molecules influence coefficients to considering processes.

Table 3. Coefficients of signal molecules mutual influence

Coefficient	Influence	Coefficient value
k_4^2	IL-1α/IL-1β на BMP-7	0,65
k_3^2	TNF-α на BMP-7	0,33
k_4^3	IL-1α/IL-1β на TNF-α	3
k_4^1	IL-1α/IL-1β на TGF-β	1,5
k_3^1	TNF-α на TGF-β	3
k_3^4	TNF-α на IL-1α/IL-1β	10

Consequently, considering values of Table 2 and Table 3, the set of equations (2) turns up at the following form:

$$(3)\ \begin{cases} M(t+\Delta t) = M(t)(1{,}23 + \dfrac{0{,}00017u_2(t)}{u_3(t)u_4(t)} - 0{,}02u_3(t)u_4(t)); \\ C(t+\Delta t) = C(t)(0{,}943 + 0{,}012u_1(t)u_3(t)u_4(t) - 0{,}069u_3(t)u_4(t)). \end{cases}$$

Now we attempt to use this set of equations to predict the state of considering tissue engineering system. Consider the following initial conditions: 1) scaffold remodeling into native matrix completed at 50 %; 2) chondrocytes volume ratio is 2 %. At the time t^0 there are activations of TGF-β in concentration of 30 ng/ml and BMP-7 in concentration of 3 mkg/ml. Cytokines concentration is near the physiological standard (suppose it is 2,56 ng/ml). Then there is the following cartilage state forecast:

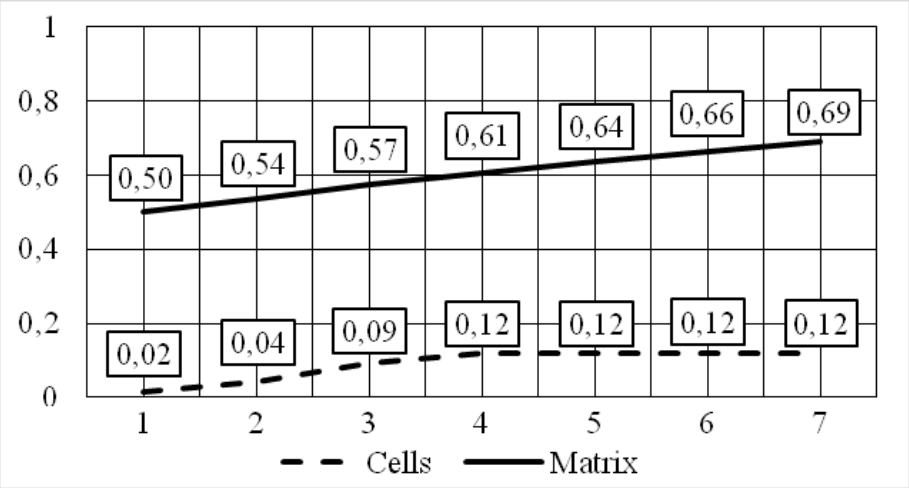

Fig. 4. Scaffold remodeling forecast (weeks 1–6)

It is apparent that TGF emission boosts chondrocytes proliferation and differentiation – in 4 weeks after its activation their volume ratio at the remodeling zone reaches the physiological limit 12 %. So, they also boost extracellular matrix synthesis. In 12 weeks, signal molecules concentrations return to normal and the system shifts to stationary state.

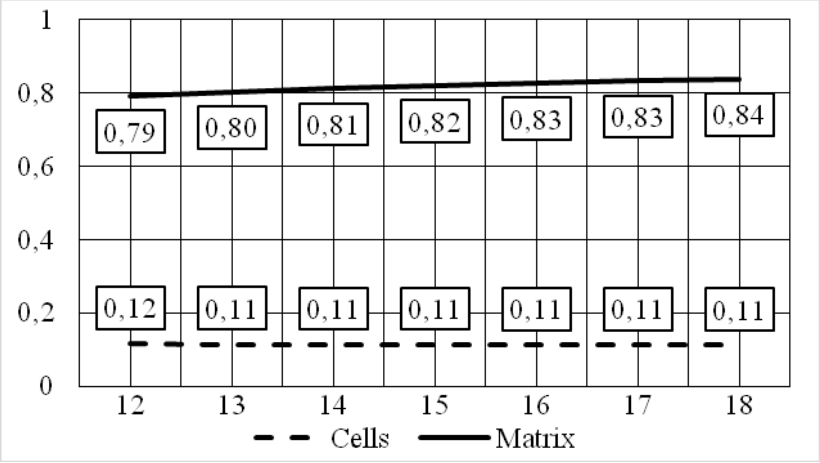

Fig. 5. The stationary state of system "Cartilage – Scaffold" (weeks 12–18)

4. Conclusion

The proposed model contains a lot of assumptions and simplifications. It does not consider structural and functional characteristics of cartilage at surface and at the deep zone, at stressed and non-stressed areas. The set of controlling actions does not contain at least four molecules which

have proven influence to considering processes. The fact that processes flow rate depends from concentrations of corresponding signal molecules non-linearly also left out of consideration.

Nevertheless, forecasted values obtained during the simulation are in physiological intervals. It indicates that the proposed approach is promising and it is reasonable to refine the model by replacing constant coefficients with functions describing dynamics of actual biological processes. As the result, we expect to obtain a model suitable not only for forecast, but also for inverse solution, which will open door to development new generation of tissue engineering implants with predefined and controlled characteristics. Such implants can be produced using various bioprinting technology, which allows scaffolds to meet the defect-specific requirements (Li et al., 2016).

Bioprinting technology shows potential in tissue engineering for the fabrication of scaffolds, cells, tissues and organs reproducibly and with high accuracy. Bioprinting technologies are mainly divided into three categories, inkjet-based bioprinting, pressure-assisted bioprinting and laser-assisted bioprinting, based on their underlying printing principles. These various printing technologies have their advantages and limitations. Bioprinting utilizes biomaterials, cells or cell factors as a "bioink" to fabricate prospective tissue structures. Biomaterial parameters such as biocompatibility, cell viability and the cellular microenvironment strongly influence the printed product. Various printing technologies have been investigated, and great progress has been made in printing various types of tissue, including vasculature, heart, bone, cartilage, skin and liver. This review introduces basic principles and key aspects of some frequently used printing technologies. We focus on recent advances in three-dimentional printing applications, current challenges and future directions (Li et al., 2016).

References

Almalki, 2016 – *Almalki S.G., Agrawal D.K.* (2016). Effects of matrix metalloproteinases on the fate of mesenchymal stem cells. *Stem Cell Res. Ther.* 7(1), e129. doi: 10.1186/s13287-016-0393-1

Asanbaeva et al., 2008 – *Asanbaeva A., Masuda K., Thonar E.J.-M.A., et al.* (2008). Regulation of immature cartilage growth by IGF-1, TGF-β1, BMP-7, and PDGF-AB: role of metabolic balance between fixed charge and collagen network. *Biomech. modeling Mechanobiol.* 7(4), pp. 263-276.

Bogatov et al., 2015 – *Bogatov V.B., Zeinalov P.V., Liubun' G.P., et al.* (2015). Remodeling of the articular cartilage during the replacement of its defect by a biocomposite material. *Morphology (SPb).* 147(1), pp. 63-69. [in Rus., Eng. abstr.]

Bhardwaj et al., 2011 – *Bhardwaj N., Nguyen Q.T., Chen A.C., et al.* (2011). Potential of 3-D tissue constructs engineered from bovine chondrocytes/silk fibroin-chitosan for in vitro cartilage tissue engineering. *Biomaterials.* 32(25), pp. 5773-5781. DOI: 10.1016/j.biomaterials. 2011.04.061

Correia et al., 2011 – *Correia C.R., Moreira-Teixeira L.S., Moroni L., et al.* (2011). Chitosan scaffolds containing hyaluronic acid for cartilage tissue engineering. Tissue Eng. Part C. Methods. 17(7), pp. 717-730. DOI: 10.1089/ten.tec.2010.0467

da Silva et al., 2010 – *da Silva A.M.L., Crawford A., Mundy J.M., et al.* (2010). Chitosan-polyester-based scaffolds for cartilage tissue engineering: assessment of extracellular matrix formation. Acta Biomater. 6(3), pp. 1149–1157. DOI: 10.1016/j.actbio.2009.09.006.

de Isla et al., 2010 – *de Isla N., Huseltein C., Jessel N., et al.* (2010). Introduction to tissue engineering and application for cartilage engineering. *Biomed. Mater. Eng.* 20(3), pp. 127-133. doi: 10.3233/BME-2010-0624

Elder, 2009 – *Elder B.D., Athanasiou K.A.* (2009). Systematic assessment of growth factor treatment on biochemical and biomechanical properties of engineered articular cartilage constructs. *Osteoarthritis Cartilage.* 17(1), pp. 114–123. doi: 10.1016/j.joca.2008.05.006

Fitzpatrick, 2015 – *Fitzpatrick L E., McDevitt T.C.* (2015). Cell-derived matrices for tissue engineering and regenerative medicine applications. *Biomater. Sci.* 3(1), pp. 12–24. doi: 10.1039/C4BM00246F

Getgood et al., 2009 – *Getgood A., Brooks R., Fortier L., Rushton N.* (2009). Articular cartilage tissue engineering: today's research, tomorrow's practice? *J. Bone Joint Surg. Br.* 91(5), pp. 565–576.

Giorgi et al., 2016 – *Giorgi M., Verbruggen S.W., Lacroix D.* (2016). In silico bone mechanobiology: modeling a multifaceted biological system. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 8(6), pp. 485–505. doi: 10.1002/wsbm.1356

Goldring, 2012 – *Goldring M.B.* (2012). Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. Ther. Adv. Musculskel. Dis. 4(4), pp. 269-285. doi: 10.1177/1759720X12448454

Gómez-Leduc et al., 2016 – *Gómez-Leduc T., Hervieu M., Legendre F., et al.* (2016). Chondrogenic commitment of human umbilical cord blood-derived mesenchymal stem cells in collagen matrices for cartilage engineering. *Sci. Rep.* 6, p. e32786. doi: 10.1038/srep32786

Hiemer et al., 2016 – *Hiemer B., Genz B., Jonitz-Heincke A., et al.* (2016) Devitalisation of human cartilage by high hydrostatic pressure treatment: Subsequent cultivation of chondrocytes and mesenchymal stem cells on the devitalized tissue. *Sci. Rep.* 6, e33747. doi: 10.1038/srep33747

Hunziker, 2009 – *Hunziker, E.B.* (2009). The elusive path to cartilage regeneration. *Adv. Mater.* 21(32-33), pp. 3419–3424. doi: 10.1002/adma.200801957

Ivanov et al., 2015 – *Ivanov A.N., Kozadaev M.N., Bogomolova N.V., et al.* (2015). Biocompatibility of polycaprolactone and hydroxyapatite matrices in vivo. *Cell Tissue Biol.* 9(5), pp. 422-429. doi: 10.1134/S1990519X15050077

Kuo, 2011 – *Kuo Y.C., Wang C.C.* (2011). Surface modification with peptide for enhancing chondrocyte adhesion and cartilage regeneration in porous scaffolds. *Colloids Surf. B. Biointerfaces.* 84(1), pp. 63-70. doi: 10.1016/j.colsurfb.2010.12.021

Li et al., 2016 – *Li J., Chen M., Fan X., Zhou H.* (2016). Recent advances in bioprinting techniques: approaches, applications and future prospects. *J. Transl. Med.* 14, e271. doi: 10.1186/s12967-016-1028-0

Lu et al., 2013 – *Lu T., Li Y., Chen T.* (2013). Techniques for fabrication and construction of three-dimensional scaffolds for tissue engineering. *Int. J. Nanomedicine.* 8, pp. 337–350. DOI: 10.2147/IJN.S38635

Maitz, 2015 – *Maitz M.F.* (2015). Applications of synthetic polymers in clinical medicine. *Biosurf. Biotribol.* 1(3), pp. 161–176.

Malafaya et al., 2010 – *Malafaya P.B., Oliveira J.T., Reis R.L.* (2010). The effect of insulin-loaded chitosan particle-aggregated scaffolds in chondrogenic differentiation. *Tissue Eng. Part A.* 16(2), pp. 735-747.

Mao, 2015 – *Mao A.S., Mooney D.J.* (2015). Regenerative medicine: Current therapies and future directions. *Proc. Natl. Acad. Sci. U. S. A.* 112(47), pp. 14452–14459. doi: 10.1016/j.bsbt.2015.08.002

McNary et al., 2012 – *McNary S.M., Athanasiou K.A., Reddi A.H.* (2012). Engineering lubrication in articular cartilage. *Tissue Engineering Pt B: Rev.* 18 (2), pp. 88-100. DOI: 10.1089/ten.teb.2011.0394.

Novochadov, 2013 – *Novochadov V.V.* (2013). Growth factor technologies in cartilage tissue engineering (review). *Eur. J. Mol. Biotech.* 1(1), pp. 28-37. doi: 10.13187/ejmb.2013.1.4

O'Brien, 2011 – *O'Brien F.J.* (2011) Biomaterials and scaffolds for tissue engineering. *Mater. Today.* 14, pp. 88–95. doi: 10.1016/S1369-7021(11)70058-X

Riera et al., 2011 – *Riera K.M., Rothfusz N.E., Wilusz R.E., et al.* (2011). Interleukin-1, tumor necrosis factor-α, and transforming growth factor-β1 and integrative meniscal repair: influences on meniscal cell proliferation and migration. *Arthritis Res. Ther.* 13(6), R187. doi:10.1186/ar3515.

Shiroky, 2014 – *Shiroky A.A., Volkov A.V., Novochadov V.V.* (2014). Crucial processes' interaction during the renewal of articular cartilage: the mathematical modeling. *Eur. J. Mol. Biotech.* 4(2), pp. 86-94. doi: 10.13187/ejmb.2014.4.86

van Osch et al., 2009 – *van Osch G.J., Brittberg M., Dennis J.E., et al.* (2009). Cartilage repair: past and future – lessons for regenerative medicine. *J. Cell Mol. Med.* 13(5), pp. 792–810. doi:10.1111/j.1582-4934.2009.00789.x

Zhang et al., 2009 – *Zhang L., Hu J., Athanasiou K.A.* (2009). The role of tissue engineering in articular cartilage repair and regeneration. *Crit. Rev. Biomed. Eng.* 37(1-2), pp. 1–57.

Zhen, 2014 – *Zhen G., Cao X.* (2014). Targeting TGFβ signaling in subchondral bone and articular cartilage homeostasis. *Trends Pharmacol. Sci.* 35(5), pp. 227-236. DOI: 10.1016/j.tips.2014.03.005

Zohreh et al., 2012 – *Zohreh I., Xiongbiao C., William K.* (2012). Strategic design and fabrication of engineered scaffolds for articular cartilage repair. *J. Funct. Biomater.* 3(4), pp. 799–838. DOI:10.3390/jfb3040799

Управление ремоделированием тканеинженерных конструкций, применяемых для лечения остеоартроза

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Аннотация. Значительный прогресс технологий тканевой инженерии и регенеративной медицины технологии (TERM-технологий) в настоящее время связывается с использованием высокоточных, преимущественно малоинвазивных методов лечения травм и хронических заболеваний суставов. Сущность тканевой инженерии суставов состоит в разработке и производстве биоинженерных матриц (скаффолдов) и последующей их имплантации в бесклеточном варианте, или предварительно заселенных подходящим пулом клеток для восстановления дефектов полноценно трехмерно-организованной тканью. Важной задачей при этом подходе является определенная индивидуализация свойств скаффолда, которая на настоящий момент практически не реализуется в направлении соответствия свойствам хряща конкретного пациента.

Основной целью исследования является описание тканеинженерной системы “хрящ – скаффолд” с использованием системной биологии и биокибернетического подхода. Задача состоит в том, чтобы спрогнозировать развитие рассматриваемой системы во времени и исследовать возможности этой системы и решить проблему управления, которая может создать возможности для создания скаффолдов с заранее заданными свойствами. Авторы рассмотрели упомянутые тканеинженерные системы как системы с управляемой обратной связью и предложили систему разностных уравнений, описывающих ее динамику.

Результаты компьютерного моделирования и прогнозные значения соотношения объема клеток и внеклеточного матрикса в физиологический промежуток времени, необходимого для ремоделирования скаффолда, в первом приближении совпадают с полученными ранее экспериментальными данными. Следующий шаг состоит в изменении модели для решения обратной задачи – разработки нового поколения тканеинженерных имплантатов с заранее заданными и контролируемыми свойствами.

Ключевые слова: математическое моделирование, биологические системы, суставной хрящ, остеоартроз, тканевая инженерия, регенеративная медицина.

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